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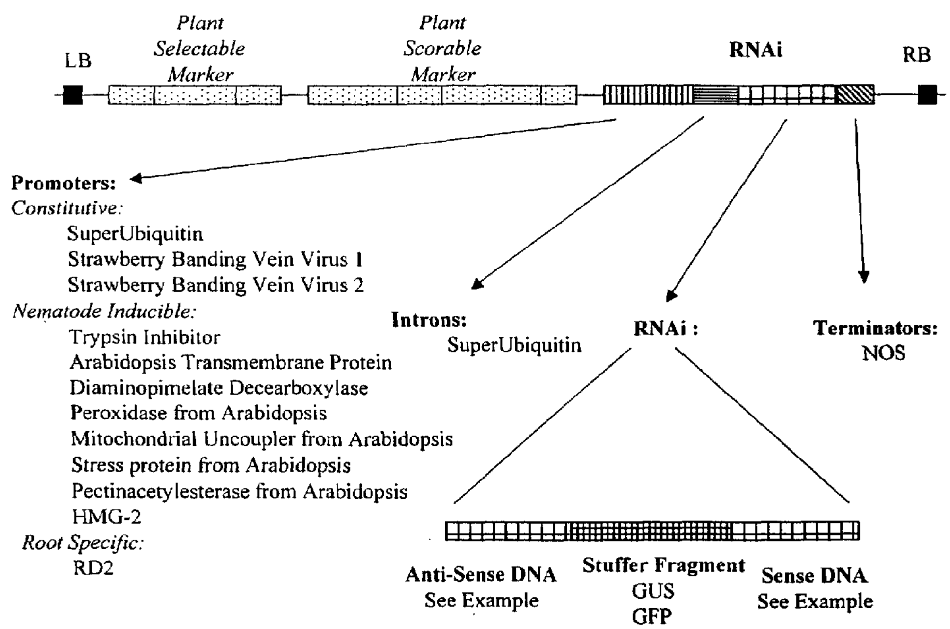
(54) Title: MATERIALS AND METHODS FOR THE CONTROL OF NEMATODES



(57) **Abstract:** The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides RNAi molecules, polynucleotide sequences, and methods of using these sequences in nematode control.

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DESCRIPTION

MATERIALS AND METHODS FOR THE CONTROL OF NEMATODES

Background of the Invention

[0001] Plant parasitic nematodes, such as root-knot nematodes *(Meloidogyne* species) and cyst nematodes (*Globodera* and *Heterodera*), attack nearly every food crop, and are among the world's most damaging agricultural pests. For example, root-knot nematodes parasitize more than 2,000 plant species from diverse plant families and represent a tremendous threat to crop production world-wide. These biotrophic pathogens have evolved highly specialized and complex feeding relationships with their hosts.

[0002] Nematodes cause millions of dollars of damage each year to turf grasses, ornamental plants, and food crops. Efforts to eliminate or minimize damage caused by nematodes in agricultural settings have typically involved the use of soil fumigation with materials such as chloropicrin, methyl bromide, and dazomet, which volatilize to spread the active ingredient throughout the soil. Such fumigation materials can be highly toxic and may create an environmental hazard. Various non-fumigant chemicals have also been used, but these too create serious environmental problems and can be highly toxic to humans.

[0003] Some research articles have been published concerning the effects of δ-endotoxins from *B. thuringiensis* species on the viability of nematodes. See, for example, Bottjer, Bone and Gill ([1985] *Experimental Parasitology* 60:239-244); Ignoffo and Dropkin (Ignoffo, C.M., Dropkin, V.H. [1977] *J. Kans. Entomol. Soc.* 50:394-398); and Ciordia, H. and W.E. Bizzell ([1961] *Jour. of Parasitology* 47:41 [abstract]). Several patents have issued describing the control of nematodes with *B.t.* See, for example, U.S. Patent Nos. 4,948,734; 5,093,120; 5,281,530; 5,426,049; 5,439,881; 5,236,843; 5,322,932; 5,151,363; 5,270,448; 5,350,577; 5,667,993; and 5,670,365. The development of resistance by insects to *B.t.* toxins is one obstacle to the successful use of such toxins.

[0004] The pesticidal activity of avermectins is well known. The avermectins are disaccharide derivatives of pentacyclic, 16-membered lactones. They can be divided into four major compounds: A_{1a}, A_{2a}, B_{1a}, and B_{2a}; and four minor compounds: A_{1b}, A_{2b}, B_{1b}, and B_{2b}. The isolation and purification of these compounds is also described in U.S. Patent No. 4,310,519, issued January 12, 1982. Avermectin B_{2a} is active against the root-knot nematode, *Meloidogyne incognita*. It is reported to be 10-30 times as potent as commercial contact nematicides when incorporated into soil at 0.16-0.25 kg/ha (Boyce Thompson Institute for Plant Research 58th Annual Report [1981]; Putter, I. *et al.* [1981] "Avermectins: Novel Insecticides, Acaracides, and Nematicides from a Soil Microorganism," *Experientia* 37:963-964). Avermectin B_{2a} is not toxic to tomatoes or cucumbers at rates of up to 10 kg/ha.

[0005] Fatty acids are a class of natural compounds which occur abundantly in nature and which have interesting and valuable biological activities. Tarjan and Cheo (Tarjan, A.C., P.C. Cheo [1956] "Nematocidal Value of Some Fatty Acids," Bulletin 332, Contribution 884, Agricultural Experiment Station, University of Rhode Island, Kingston, 41 pp.) report the activity of certain fatty acids against nematodes. In 1977 Sitaramaiah and Singh (Sitaramaiah, K., R.S. Singh [1977] *Indian J. Nematol.* 7:58-65) also examined the response of nematodes to fatty acids. The results of these tests with short chain acids were equivocal, showing nematode-inhibitory action in some instances and stimulatory activity in other instances. Phytotoxicity of these acids was observed at higher concentrations. The short chain fatty acids were also examined by Malik and Jairajpuri (Malik, Z., M.S. Jairajpuri [1977] *Nematol. medit.* 12:73-79), who observed nematode toxicity at high concentrations of the fatty acids.

[0006] Notwithstanding the foregoing (some of the limitations of and problems associated with these approaches are discussed above), there is a need for safe and effective alternatives for controlling nematodes.

[0007] One method for disrupting normal cellular processes is by the use double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). When RNAi corresponding to a sense and antisense sequence of a target mRNA is introduced into a cell, the targeted mRNA is degraded and protein translation of that message is stopped. Although not yet fully understood, the mechanism of this post-transcriptional gene

silencing appears to be at least partially due to the generation of small RNA molecules, about 21 - 25 nucleotides in length, that correspond to the sense and antisense pieces of the RNAi introduced into the cell (Bass, B. L. [2000] "Double-stranded RNA as a template for gene silencing" *Cell* 101:235-238).

[0008] The specificity of this gene silencing mechanism appears to be extremely high, blocking expression only of targeted genes, while leaving other genes unaffected. A recent example of the use of RNAi; to inhibit genetic function in plants used *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* (Chuang, C.-F. and E. M. Meyerowitz [2000] "Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*" *Proc. Natl. Acad. Sci. USA* 97:4985-4990). Chuang *et al.* describe the construction of vectors delivering variable levels of RNAi targeted to each of four genes involved in floral development. Severity of abnormal flower development varied between transgenic lines. For one of the genes, AGAMOUS (AG), a strong correlation existed between declining accumulation of mRNA and increasingly severe phenotypes, suggesting that AG-specific endogenous mRNA is the target of RNAi.

Brief Summary of the Invention

[0009] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences that encode nematode genes, RNAi that selectively targets mRNA transcripts of these essential nematode genes, and methods of using these sequences in nematode control strategies. Such sequences for use according to the subject invention are summarized in Appendix 1. RNAi molecules disclosed herein can be used to inhibit the expression of one or more of these genes in nematodes.

Brief Description of the Drawings

[00010] **Figure 1**: Modular Binary Construct System (MBCS): A series of six, 8-base cutter restriction enzyme sites has been placed between the left and right Ti borders of a previously created kan^R/tet^R binary plasmid.

[00011] **Figure 2**: An exemplary shuttle vector created for cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites.

[00012] **Figure 3**: An exemplary shuttle vector with exemplary inserts.

[00013] **Figure 4**: A suggested RNAi binary vector with exemplary inserts.

[00014] **Figure 5**: Exemplary selectable markers for MBCS.

[00015] **Figure 6**: Exemplary scorable markers for MCBS.

[00016] **Figure 7**: Exemplary RNAi binary vector.

[00017] **Figure 8**: Exemplary RNAi shuttle vector.

Brief Description of the Sequences

[00018] Brief Description of the Sequences can be found in Appendix I.

Detailed Disclosure of the Invention

[00019] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences and methods of using these sequences in nematode control strategies. A preferred method for controlling nematodes according to the subject invention provides materials and methods for controlling nematodes by using double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). The terms RNAi and RNAi are used interchangeably herein unless otherwise noted.

[00020] In one embodiment of the invention, RNAi molecules are provided which are useful in methods of killing nematodes and/or inhibiting their growth, development, parasitism or reproduction. RNAi molecules of the invention are also useful for the regulation of levels of specific mRNA in nematodes.

[00021] dsRNA (RNAi) typically comprises a polynucleotide sequence identical to a target gene (or fragment thereof) linked directly, or indirectly, to a polynucleotide

sequence complementary to the sequence of the target gene (or fragment thereof). The dsRNA may comprise a polynucleotide linker (stuffer) sequence of sufficient length to allow for the two polynucleotide sequences to fold over and hybridize to each other; however, a linker sequence is not necessary. The linker (stuffer) sequence is designed to separate the antisense and sense strands of RNAi significantly enough to limit the effects of steric hindrances and allow for the formation of dsRNA molecules.

[00022] RNA containing a nucleotide sequence identical to a fragment of the target gene is preferred for inhibition; however, RNA sequences with insertions, deletions, and point mutations relative to the target sequence can also be used for inhibition. Sequence identity may optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, *Sequence Analysis Primer*, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (*e.g.*, University of Wisconsin Genetic Computing Group). Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a fragment of the target gene transcript.

[00023] As disclosed herein, 100% sequence identity between the RNA and the target gene is not required to practice the present invention. Thus the invention has the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence.

[00024] RNA may be synthesized either *in vivo* or *in vitro*. Endogenous RNA polymerase of the cell may mediate transcription *in vivo*, or cloned RNA polymerase can be used for transcription *in vivo* or *in vitro*. For transcription from a transgene *in vivo* or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands). Inhibition may be targeted by specific transcription in an organ, tissue, or cell type; stimulation of an environmental condition (e.g., infection, stress, temperature, chemical inducers); and/or engineering transcription at a developmental stage or age. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. RNA

may be chemically or enzymatically synthesized by manual or automated reactions. The RNA may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (e.g., T3, T7, SP6). The use and production of an expression construct are known in the art (see, for example, WO 97/32016; U.S. Pat. Nos. 5,593,874; 5,698,425; 5,712,135; 5,789,214; and 5,804,693; and the references cited therein). If synthesized chemically or by *in vitro* enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA may be used with no or a minimum of purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

[00025] Preferably and most conveniently, RNAi can be targeted to an entire polynucleotide sequence of a gene set forth herein. Preferred RNAi molecules of the instant invention are highly homologous or identical to the polynucleotides summarized in Appendix 1. The homology is preferably greater than 90% and is most preferably greater than 95%.

[00026] Fragments of genes can also be targeted. These fragments are typically in the approximate size range of about 20 nucleotides. Thus, targeted fragments are preferably at least about 15 nucleotides. In certain embodiments, the gene fragment targeted by the RNAi molecule is about 20-25 nucleotides in length. However, other size ranges can also be used. For example, using a *C. elegans* microinjection assay, RNAi "fragments" of about 60 nucleotides with between 95 and 100% identity (to a nematode gene) were determined to cause excellent inhibition.

[00027] Thus, RNAi molecules of the subject invention are not limited to those that are targeted to the full-length polynucleotide or gene. The nematode gene product can be inhibited with a RNAi molecule that is targeted to a portion or fragment of the exemplified polynucleotides; high homology (90-95%) or identity is also preferred, but not necessarily essential, for such applications.

[00028] The polynucleotide sequences identified in Appendix A and shown in the Sequence ID listing are from genes encoding nematode proteins having the functions

shown in Appendix 1. The genes exemplified herein are representative of particular classes of proteins which are preferred targets for disruption according to the subject invention. These classes of proteins include, for example, proteins involved in ribosome assembly; neurol transmitter receptors and ligands; electron transport proteins; metabolic pathway proteins; and protein and polynucleotide production, folding, and processing proteins.

[00029] Genetic regulatory sequences, such as promoters, enhancers, and terminators, can be used in genetic constructs to practice the subject invention. Such constructs themselves can also be used for nematode control. Various constructs can be used to achieve expression in specific plant tissues (by using root specific promoters, for example) and/or to target specific nematode tissues (by using targeting elements or adjacent targeting sequences, for example).

[00030] In a specific embodiment of the subject invention, plant cells, preferably root cells, are genetically modified to produce at least one RNAi that is designed to be taken up by nematodes during feeding to block expression (or the function of) of a target gene. As is known in the art, RNAi can target and reduce (and, in some cases, prevent) the translation of a specific gene product. RNAi can be used to reduce or prevent message translation in any tissue of the nematode because of its ability to cross tissue and cellular boundaries. Thus, RNAi that is contacted with a nematode by soaking, injection, or consumption of a food source will cross tissue and cellular boundaries. RNAi can also be used as an epigenetic factor to prevent the proliferation of subsequent generations of nematodes.

[00031] Nematode polynucleotide sequences disclosed herein demonstrate conserved nucleotide motifs among different nematode genera. Conserved nucleotide motifs strongly suggest that these sequences are associated with viability and/or parasitism and are functionally conserved and expressed in both *Meloidogyne incognita* (root-knot nematode) and *Globodera rostochiensis* and *Globdera pallids* (potato cyst nematodes). The use of these polynucleotides, and RNAi inhibitors thereof, is advantageous because such RNAi can be designed to have broad RNAi specificity and are thus useful for controlling a large number of plant parasitic nematodes *in planta*. Because the genes identified in this disclosure are associated with nematode survival

and/or parasitism, RNAi inhibition of these genes (arising from contacting nematodes with compositions comprising RNAi molecules) prevents and/or reduces parasitic nematode growth, development, and or parasitism.

[00032] Methods of the subject invention include the transformation of plant cells with genes or polynucleotides of the present invention, which can be used to produce nematode inhibitors or RNAi in the plants. In one embodiment, the transformed plant or plant tissue can express RNAi molecules encoded by the gene or polynucleotide sequence introduced into the plant. Other nematode inhibitors contemplated by the invention include antisense molecules specific to the polynucleotide sequences disclosed herein. The transformation of plants with genetic constructs disclosed herein can be accomplished using techniques well known to those skilled in the art and can involve modification of the gene(s) to optimize expression in the plant to be made resistant to nematode infection and infestation. Furthermore, it is known in the art that many tissues of the transgenic plants (such as the roots) can be targeted for transformation.

[00033] RNA-mediated interference (RNAi) of gene expression. Several aspects of root-knot nematode biology make classical genetic studies difficult with this organism. Since root-knot nematodes reproduce by obligatory mitotic parthenogenesis, the opportunity to perform genetic crosses is not available. Microinjection of RNAi can be used to manipulate gene expression in *C. elegans* (Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. [1998] "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*" *Nature* 391:806- 811). Microinjecting (into adult nematodes) RNAi can turn off specific genes in progeny worms complementary to the coding region of the genes. Moreover, gene inhibition occurs in progeny when RNAi is injected into the body cavity of the adult, indicating the ability of the RNAi to cross cellular boundaries. This RNAi injection method provides a molecular genetic tool that allows for analysis of gene function in root-knot nematodes.

[00034] RNAi can be taken up by *C. elegans* by simply soaking the nematodes in a solution RNAi. This results in targeted inhibition of gene expression in the nematode (Maeda, I., Y. Kohara, M. Yamamoto and A. Sugimoto [1999] "RNAi screening with a non-redundant cDNA set" International Worm Meeting, Madison, WI, abstract 565). Nematodes fed *E. coli* expressing RNAi also demonstrate targeted and

heritable inhibition of gene expression (Sarkissian, M., H. Tabara and C. C. Mello [1999] "A mut-6 screen for RNAi deficient mutants" International Worm Meeting, Madison, WI, abstract 741; Timmons, l. and A. Fire [1998] "Specific interference by ingested dsRNA" *Nature* 395:854; WO 99/32619, hereby incorporated by reference in its entirety).

[00035] Accordingly, one aspect of the instant invention is directed to the control of nematodes comprising contacting nematodes with compositions comprising RNAi molecules specific to the nematode genes disclosed herein. The contacting step may include soaking the nematodes in a solution containing RNAi molecules, feeding nematodes RNAi molecules contained in microbes or plant cells upon which the nematode feeds, or injecting nematodes with RNAi. Nematodes can also be "contacted" and controlled by RNAi expressed in plant tissues that would be consumed, ingested, or frequented by nematodes.

[00036] The RNAi molecules provided to the nematodes may be specific to a single gene. A "cocktail" of RNAi molecules specific to various segments of a single gene can also be used. In addition, a "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) may be applied to the nematodes according to the subject invention.

[00037] In addition to RNAi uptake mediated by transgenic plants, nematodes can be directly transformed with RNAi constructs of cDNAs encoding secretory or other essential proteins to reduce expression of the corresponding gene. The transgenic animals can be assayed for inhibition of gene product using immunoassays or for reduced virulence on a host. Progeny of affected worms can also be assayed by similar methods.

[00038] Procedures that can be used for the preparation and injection of RNAi include those detailed by Fire *et al.,* (1998; ftp://ciw1.ciwemb.edu). Root-knot nematodes can be routinely monoxenically cultured on *Arabidopsis thaliana* roots growing on Gamborg's B-5/Gelrite® media. This nematode-host pathosystem is ideally suited for these microinjection experiments since limited root galling results in the parasitic stages (late J2 through adult females) developing outside of the root for easy accessibility for injecting. Another advantage is the parthenogenic reproduction of root-knot nematodes, which makes fertilization by males unnecessary for egg production. The RNAi can be injected into the body cavity of parasitic stages of root-knot nematodes

feeding on *A. thaliana* roots using microinjection. Control nematodes can be injected in parallel with only buffer or an unrelated RNAi. Injected nematodes can be monitored for egg production, and the eggs can be collected for the assays described below. Female root-knot nematodes will typically survive and lay more than 250 eggs following 1 μl injection of buffer.

[00039] Alternatively, methods are available for microinjecting materials directly into the plant root cells upon which nematodes feed: giant cells or syncytial cells (Böckenhoff, A. and F.M.W. Grundler [1994] "Studies on the nutrient uptake by the beet cyst nematode *Heterodera schachtii* by *in situ* microinjection of fluorescent probes into the feeding structures in *Arabidopsis thaliana*" *Parasitology* 109:249-254). This provides an excellent test system to screen RNAi molecules for efficacy by directly inhibiting growth and development of the nematode feeding upon the microinjected plant cell, or by reducing fecundity and the ability of said nematode to generate pathogenic or viable progeny.

[00040] There are a number of strategies that can be followed to assay for RNAi gene interference. Inhibition of gene expression by RNAi inhibits the accumulation of the corresponding secretory protein in the esophageal gland cells of transgenic J2 hatched from the eggs produced by the injected nematodes. In the first assay, polyclonal antibodies to the target gene product can be used in immunolocalization studies (Hussey, R. S. [1989] "Monoclonal antibodies to secretory granules in esophageal glands of *Meloidogyne* species" *J. Nematol.* 21:392-398; Borgonie, G, E. van Driessche, C. D. Link, D. de Waele, and A. Coomans [1994] "Tissue treatment for whole mount internal lectin staining in the nematodes *Caenorhabditis elegans*, *Panagrolaimus superbus* and *Acrobeloides maximus*" *Histochemistry* 101:379-384) to monitor the synthesis of the target protein in the gland cells of progeny of the injected nematodes, or in any other nematode tissue that fails to express the essential targeted gene. Interference of endogenous gene activity by the RNAi eliminates binding of the antibodies to secretory granules in the glands, or any other target tissue, of the transgenic nematodes, and can be monitored by these *in situ* hybridization experiments. Control nematodes injected only with the injection buffer can be processed similar to the RNAi treated nematodes.

[00041] Another assay is designed to determine the effect of the RNAi on reducing the virulence of J2 progeny of the injected females. Egg masses from injected females can be transferred singly to *A. thaliana* plates to assess the ability of the transgenic J2 to infect roots. The J2 hatching from the eggs transferred to the plates can be monitored; after 25 days the number of galls with egg laying females can be recorded. The *A. thaliana* roots can also be stained with acid fuschin to enumerate the number of nematodes in the roots. Egg masses from nematodes injected only with the injection buffer can be handled similarly and used as controls. The treatments can be replicated, and the root infection data can be analyzed statistically. These experiments can be used to assess the importance of the target genes in root-knot nematode's virulence or viability. By staining the J2 progeny of the injected females with the antibodies, it can be determined whether RNAi blocks expression of the targeted gene.

[00042] <u>Additional uses of polynucleotides.</u> The polynucleotide sequences exemplified herein can be used in a variety of ways. These polynucleotides can be used in assays for additional polynucleotides and additional homologous genes, and can be used in tracking the quantitative and temporal expression of parasitism genes in nematodes. These polynucleotides can be cloned into microbes for production and isolation of their gene products. Among the many uses of the isolated gene product is the development of additional inhibitors and modifiers. The protein products of the subject polynucleotides can also be used as diagnostic tools. For example, proteins encoded by the parasitism genes, as identified herein, can be used in large scale screenings for additional peptide inhibitors. The use of peptide phage display screening is one method that can be used in this regard. Thus, the subject invention also provides new biotechnological strategies for managing nematodes under sustainable agricultural conditions.

[00043] Antisense technologies can also be used for phytopathogenic nematode control. Antisense technology can be used to interfere with expression of the disclosed endogenous nematode genes. Antisense technology can also be used to alter the components of plants used as targets by the nematodes. For example, the transformation of a plant with the reverse complement of an endogenous gene encoded by a polynucleotide exemplified herein can result in strand co-suppression and gene silencing

or inhibition of a target involved in the nematode infection process. Thus, the subject invention includes transgenic plants (which are preferably made nematode-resistant in this manner, and other organisms including microbes and phages) comprising RNAi or antisense molecules specific to any of the polynucleotides identified herein.

[00044] Polynucleotide probes. DNA possesses a fundamental property called base complementarity. In nature, DNA ordinarily exists in the form of pairs of anti-parallel strands, the bases on each strand projecting from that strand toward the opposite strand. The base adenine (A) on one strand will always be opposed to the base thymine (T) on the other strand, and the base guanine (G) will be opposed to the base cytosine (C). The bases are held in apposition by their ability to hydrogen bond in this specific way. Though each individual bond is relatively weak, the net effect of many adjacent hydrogen bonded bases, together with base stacking effects, is a stable joining of the two complementary strands. These bonds can be broken by treatments such as high pH or high temperature, and these conditions result in the dissociation, or "denaturation," of the two strands. If the DNA is then placed in conditions which make hydrogen bonding of the bases thermodynamically favorable, the DNA strands will anneal, or "hybridize," and reform the original double- stranded DNA. If carried out under appropriate conditions, this hybridization can be highly specific. That is, only strands with a high degree of base complementarity will be able to form stable double-stranded structures. The relationship of the specificity of hybridization to reaction conditions is well known. Thus, hybridization may be used to test whether two pieces of DNA are complementary in their base sequences. It is this hybridization mechanism which facilitates the use of probes of the subject invention to readily detect and characterize DNA sequences of interest.

[00045] The specifically exemplified polynucleotides of the subject invention can themselves be used as probes. Additional polynucleotide sequences can be added to the ends of (or internally in) the exemplified polynucleotide sequences so that polynucleotides that are longer than the exemplified polynucleotides can also be used as probes. Thus, isolated polynucleotides comprising one or more of the exemplified sequences are within the scope of the subject invention. Polynucleotides that have less nucleotides than the exemplified polynucleotides can also be used and are contemplated within the scope of the present invention. For example, for some purposes, it might be

useful to use a conserved sequence from an exemplified polynucleotide wherein the conserved sequence comprises a portion of an exemplified sequence. Thus, polynucleotides of the subject invention can be used to find additional, homologous (wholly or partially) genes.

[00046] Probes of the subject invention may be composed of DNA, RNA, or PNA (peptide nucleic acid). The probe will normally have at least about 10 bases, more usually at least about 17 bases, and may have about 100 bases or more. Longer probes can readily be utilized, and such probes can be, for example, several kilobases in length. The probe sequence is designed to be at least substantially complementary to a portion of a gene encoding a protein of interest. The probe need not have perfect complementarity to the sequence to which it hybridizes. The probes may be labeled utilizing techniques that are well known to those skilled in this art.

[00047] One approach for the use of the subject invention as probes entails first identifying DNA segments that are homologous with the disclosed nucleotide sequences using, for example, Southern blot analysis of a gene bank. Thus, it is possible, without the aid of biological analysis, to know in advance the probable activity of many new polynucleotides, and of the individual gene products expressed by a given polynucleotide. Such an analysis provides a rapid method for identifying commercially valuable compositions.

[00048] One hybridization procedure useful according to the subject invention typically includes the initial steps of isolating the DNA sample of interest and purifying it chemically. Either lysed nematodes or total fractionated nucleic acid isolated from nematodes can be used. Cells can be treated using known techniques to liberate their DNA (and/or RNA). The DNA sample can be cut into pieces with an appropriate restriction enzyme. The pieces can be separated by size through electrophoresis in a gel, usually agarose or acrylamide. The pieces of interest can be transferred to an immobilizing membrane.

[00049] The particular hybridization technique is not essential to the subject invention. As improvements are made in hybridization techniques, they can be readily applied.

[00050] The probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong non-covalent bond between the two molecules, it can be reasonably assumed that the probe and sample are essentially identical or very similar. The probe's detectable label provides a means for determining in a known manner whether hybridization has occurred.

[00051] In the use of the nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include ^{32}P, ^{35}S, or the like. Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or peroxidases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. In addition, the probes can be made inherently fluorescent as described in International Application No. WO 93/16094.

[00052] Various degrees of stringency of hybridization can be employed. The more stringent the conditions, the greater the complementarity that is required for duplex formation. Stringency can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170.

[00053] As used herein "moderate to high stringency" conditions for hybridization refers to conditions that achieve the same, or about the same, degree of specificity of hybridization as the conditions "as described herein." Examples of moderate to high stringency conditions are provided herein. Specifically, hybridization of immobilized DNA on Southern blots with ^{32}P-labeled gene-specific probes was performed using standard methods (Maniatis *et al.*). In general, hybridization and subsequent washes were carried out under moderate to high stringency conditions that

allowed for detection of target sequences with homology to sequences exemplified herein. For double-stranded DNA gene probes, hybridization was carried out overnight at 20-25° C below the melting temperature (Tm) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula from Beltz *et al.* (1983):

[00054] Tm=81.5°C+16.6 Log[Na+]+0.41(%G+C)-0.61(%formamide)-600/length of duplex in base pairs.

Washes are typically carried out as follows:

(1) Twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash).

(2) Once at Tm-20°C for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

[00055] For oligonucleotide probes, hybridization was carried out overnight at 10-20°C below the melting temperature (Tm) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. Tm for oligonucleotide probes was determined by the following formula from Suggs *et al.* (1981):

[00056] Tm (°C)=2(number T/A base pairs) +4(number G/C base pairs)

[00057] Washes were typically carried out as follows:

[00058] (1) Twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash).

[00059] (2) Once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

[00060] In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment of greater than about 70 or so bases in length, the following conditions can be used:

Low:	1 or 2X SSPE, room temperature
Low:	1 or 2X SSPE, 42°C
Moderate:	0.2X or 1X SSPE, 65°C
High:	0.1X SSPE, 65°C.

[00061] Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch

can be tolerated. Therefore, polynucleotide sequences of the subject invention include mutations (both single and multiple), deletions, and insertions in the described sequences, and combinations thereof, wherein said mutations, insertions, and deletions permit formation of stable hybrids with a target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence using standard methods known in the art. Other methods may become known in the future.

[00062] The mutational, insertional, and deletional variants of the polynucleotide sequences of the invention can be used in the same manner as the exemplified polynucleotide sequences so long as the variants have substantial sequence similarity with the original sequence. As used herein, substantial sequence similarity refers to the extent of nucleotide similarity that is sufficient to enable the variant polynucleotide to function in the same capacity as the original sequence. Preferably, this similarity is greater than 50%; more preferably, this similarity is greater than 75%; and most preferably, this similarity is greater than 90%. The degree of similarity needed for the variant to function in its intended capacity will depend upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations that are designed to improve the function of the sequence or otherwise provide a methodological advantage.

[00063] <u>PCR technology</u>. Polymerase Chain Reaction (PCR) is a repetitive, enzymatic, primed synthesis of a nucleic acid sequence. This procedure is well known and commonly used by those skilled in this art (see U.S. Patent Nos. 4,683,195; 4,683,202; and 4,800,159; Saiki *et al.*, 1985). PCR is based on the enzymatic amplification of a DNA fragment of interest that is flanked by two oligonucleotide primers that hybridize to opposite strands of the target sequence. The primers are oriented with the 3′ ends pointing towards each other. Repeated cycles of heat denaturation of the template, annealing of the primers to their complementary sequences, and extension of the annealed primers with a DNA polymerase result in the amplification of the segment defined by the 5′ ends of the PCR primers. Since the extension product of each primer can serve as a template for the other primer, each cycle essentially doubles the amount of DNA fragment produced in the previous cycle. This results in the exponential accumulation of the specific target fragment, up to several million-fold in a

few hours. By using a thermostable DNA polymerase such as *Taq* polymerase, which is isolated from the thermophilic bacterium *Thermus aquaticus*, the amplification process can be completely automated. Other enzymes that can be used are known to those skilled in the art.

[00064] The polynucleotide sequences of the subject invention (and portions thereof such as conserved regions and portions that serve to distinguish these sequences from previously-known sequences) can be used as, and/or used in the design of, primers for PCR amplification. In performing PCR amplification, a certain degree of mismatch can be tolerated between primer and template. Therefore, mutations, deletions, and insertions (especially additions of nucleotides to the 5′ end) of the exemplified ppolynucleotides can be used in this manner. Mutations, insertions and deletions can be produced in a given primer by methods known to an ordinarily skilled artisan.

[00065] The polynucleotide sequences of the instant invention may be "operably linked" to regulatory sequences such as promoters and enhancers. Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is "operably linked" to DNA encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is "operably linked" to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is "operably linked" to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[00066] <u>Polynucleotides and proteins</u>. Polynucleotides of the subject invention can be defined according to several parameters. One characteristic is the biological activity of the protein products as identified herein. The proteins and genes of the subject invention can be further defined by their amino acid and nucleotide sequences. The sequences of the molecules can be defined in terms of homology to certain exemplified sequences as well as in terms of the ability to hybridize with, or be amplified by, certain

exemplified probes and primers. Additional primers and probes can readily be constructed by those skilled in the art such that alternate polynucleotide sequences encoding the same amino acid sequences can be used to identify and/or characterize additional genes. The proteins of the subject invention can also be identified based on their immunoreactivity with certain antibodies.

[00067] The polynucleotides and proteins of the subject invention include portions, fragments, variants, and mutants of the full-length sequences as well as fusions and chimerics, so long as the encoded protein retains the characteristic biological activity of the proteins identified herein. As used herein, the terms "variants" or "variations" of genes refer to nucleotide sequences that encode the same proteins or which encode equivalent proteins having equivalent biological activity. As used herein, the term "equivalent proteins" refers to proteins having the same or essentially the same biological activity as the exemplified proteins.

[00068] It will be apparent to a person skilled in this art that genes within the scope of the subject invention can be identified and obtained through several means. The specific genes exemplified herein may be obtained from root-knot nematodes. Genes, or portions or variants thereof, may also be artificially synthesized by, for example, a gene synthesizer.

[00069] Variations of genes may be readily constructed using standard techniques such as site-directed mutagenesis and other methods of making point mutations and by DNA shuffling, for example. In addition, gene and protein fragments can be made using commercially available exonucleases, endonucleases, and proteases according to standard procedures. For example, enzymes such as *Bal*31 can be used to systematically cut off nucleotides from the ends of genes. In addition, genes that encode fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these proteins. Of course, molecular techniques for cloning polynucleotides and producing gene constructs of interest are also well known in the art. *In vitro* evaluation techniques, such as MAXYGEN's "Molecular Breeding" can also be applied to practice the subject invention.

[00070] Other molecular techniques can also be applied using the teachings provided herein. For example, antibodies raised against proteins encoded by

polynucleotides disclosed herein can be used to identify and isolate proteins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the proteins that are conserved and most distinct from other proteins. These antibodies can then be used to specifically identify equivalent proteins by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to proteins encoded by polynucleotides disclosed herein, or to equivalent proteins, can readily be prepared using standard procedures known in the art. The genes that encode these proteins can be obtained from various organisms.

[00071] Because of the redundancy of the genetic code, a variety of different DNA sequences can encode the amino acid sequences encoded by the polynucleotide sequences disclosed herein. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding proteins having the same, or essentially the same, amino acid sequence. These variant DNA sequences are within the scope of the subject invention. As used herein, reference to "essentially the same" sequence refers to sequences that have amino acid substitutions, deletions, additions, or insertions that do not materially affect biological activity. Fragments retaining the characteristic biological activity are also included in this definition.

[00072] A further method for identifying genes and polynucleotides (and the proteins encoded thereby) of the subject invention is through the use of oligonucleotide probes. Probes provide a rapid method for identifying genes of the subject invention. The nucleotide segments that are used as probes according to the invention can be synthesized using a DNA synthesizer and standard procedures.

[00073] The subject invention comprises variant or equivalent proteins (and nucleotide sequences coding for equivalent proteins or for inhibitors of the genes encoding such proteins) having the same or similar biological activity of inhibitors or proteins encoded by the exemplified polynucleotides. Equivalent proteins will have amino acid similarity with an exemplified protein (or peptide). The amino acid and/or nucleotide identity will typically be greater than 60%. Preferably, the identity will be greater than 75%. More preferably, the identity will be greater than 80%, and even more preferably greater than 90%. Most preferably, the identity will be greater than 95%. RNAi molecules will also have corresponding identities in these preferred ranges. These

identities are as determined using standard alignment techniques for determining amino acid and/or nucleotide identity. The identity/similarity will be highest in critical regions of the protein or gene including those regions that account for biological activity or that are involved in the determination of three-dimensional configuration that is ultimately responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Below is a list of examples of amino acids belonging to various classes

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

[00074] In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not detract from the ability to manage nematode-caused diseases.

[00075] An "isolated" or "substantially pure" nucleic acid molecule or polynucleotide is a polynucleotide that is substantially separated from other polynucleotide sequences which naturally accompany a nucleic acid molecule. The term embraces a polynucleotide sequence which was removed from its naturally occurring environment by the hand of man. This includes recombinant or cloned DNA isolates,

chemically synthesized analogues and analogues biologically synthesized by heterologous systems. An "isolated" or "purified" protein, likewise, is a protein removed from its naturally occurring environment.

[00076] Recombinant hosts. The genes, antisense, and RNAi polynucleotides within the scope of the present invention can be introduced into a wide variety of microbial or plant hosts. Plant cells can be transformed (made recombinant) in this manner. Microbes, for example, can also be used in the application of RNAi molecules of the subject invention in view of the fact that microbes are a food source for nematodes

[00077] There are many methods for introducing a heterologous gene or polynucleotide into a host cell or cells under conditions that allow for stable maintenance and expression of the gene or polynucleotide. These methods are well known to those skilled in the art. Synthetic genes, such as, for example, those genes modified to enhance expression in a heterologous host (such as by preferred codon usage or by the use of adjoining, downstream, or upstream enhancers) that are functionally equivalent to the genes (and which encode equivalent proteins) can also be used to transform hosts. Methods for the production of synthetic genes are known in the art.

[00078] Where the gene or polynucleotide of interest is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, certain host microbes are preferred. Certain microorganism hosts are known to occupy the phytosphere, phylloplane, phyllosphere, rhizosphere, and/or rhizoplane of one or more crops of interest. These microorganisms can be selected so as to be capable of successfully competing in the particular environment (crop and other habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing a polypeptide of interest, and, desirably, provide for improved protection of the protein/peptide from environmental degradation and inactivation.

[00079] A large number of microorganisms is known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, *e.g.*, genera *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilius*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*,

Arthrobacter, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, *e.g.*, genera *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are the pigmented microorganisms.

[00080] Methods of the subject invention also include the transformation of plants or plant tissue with genes which encode the RNAi molecules of the present invention. In one embodiment, the transformed plant or plant tissue expresses antisense RNA and/or RNAi. Transformation of cells can be made by those skilled in the art using standard techniques. Materials necessary for these transformations are disclosed herein or are otherwise readily available to the skilled artisan.

[00081] Additional methods and formulations for control of pests. Control of nematode pests using the RNAi molecules of the instant invention can be accomplished by a variety of additional methods that would be apparent to those skilled in the art having the benefit of the subject disclosure. A "cocktail" of two or more RNAi molecules can be used to disrupt one or more of the genes identified herein. The "cocktail" of RNAi molecules may be specific to segments of a single gene or the entire gene. A "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) is also encompassed by the instant invention. In another embodiment of the instant invention, the disclosed RNAi molecules, cocktails, and/or multigene cocktails thereof, may be used in conjunction with other known nematode control agents and methodologies. Such cocktails can be used to combat the development of resistance by nematodes to a certain inhibitor or inhibitors.

[00082] Compositions of the subject invention which comprise RNAi molecules and carriers can be applied, themselves, directly or indirectly, to locations frequented by, or expected to be frequented by, nematodes. Microbial hosts which were transformed with polynucleotides that encode RNAi molecules, express said RNAi molecules, and which colonize roots (*e.g.*, *Pseudomonas*, *Bacillus*, and other genera) can be applied to the sites of the pest, where they will proliferate and be ingested. The result is control of the pest. Thus, methods of the subject invention include, for example, the application of recombinant microbes to the pests (or their locations). The recombinant microbes may also be transformed with more than one RNAi molecule thereby delivering a "cocktail" of RNAi molecules to the nematode pests. A carrier may be any substance suitable for

delivering the RNAi molecules to the nematode. Acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E.W. Martin's *Remington's Pharmaceutical Science*, Mack Publishing Company, Easton, PA.

[00083] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

[00084] Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1– Production of Hairy Roots for RNAi Testing

[00085] A hairy root assay system was developed for testing the anti-nematode activity of RNAi molecules.

[00086] *Agrobacterium rhizogenes*: Several *Agrobacterium rhizogenes* strains produce hairy roots on a variety of plant species. *A. rhizogenes* strains, A4, 15834, 8196 and LBA4404 demonstrate hairy root development on tomato and sugar beet, with A4 being the most efficient. The *A. rhizogenes* strain K599 demonstrated very efficient formation on transgenic soybean hairy roots and was also effective on sugar beet and *Arabidopsis*. However, stain K599 failed to produce hairy roots on tomato tissues possibly due to hyper-virulence.

[00087] Hairy root production: Transgenic hairy roots were identified by stable GUS expression in tomato, sugar beet, soybean and *Arabidopsis*. The construct pAKK1401 (pNOS / NPT-II / tNOS // pSU / GUS / tNOS) was used to produce hairy roots when transformed into *A. rhizogenes* strains A4 or K599. Transgenic roots were identified by GUS expression.

Example 2 – Protocol for Electro-competent *Agrobacterium* and Electroporation

[00088] Electro-competent Agrobacterium Protocol:

[00089] 1. Grow *Agrobacterium* overnight in 5 mls LB + antibiotics at 30°C on shaker (for *Agrobacterium rhizogenes* strain K599 no antibiotics are needed).

[00090] 2. Use the 5 mls of overnight culture to inoculate 500 mls LB + antibiotics at 30°C on shaker. Grow overnight.

[00091] 3. Add liquid culture in eight 50 ml polypropylene orange cap tubes.

[00092] 4. Centrifuge 10 min., 4000 rpm, 4°C.

[00093] 5. Resuspend cells in each tube with 20 mls 10% glycerol (on ice)

[00094] 6. Centrifuge 10 min., 4000 rpm, 4°C.

[00095] 7. Resuspend cells in each tube with 10 mls 10% glycerol (on ice).

[00096] 8. Centrifuge 10 min., 4000 rpm , 4°C.

[00097] 9. Resuspend cells in each tube with 2 mls 10% glycerol (on ice).

[00098] 10. Aliquot 50 µl into cold Eppendorf tube and place onto dry ice.

[00099] 11. Store electro-competent cells at -80°C. These cells can be used for up to two years.

[000100] Electroporations:

[000101] 1. Add 1 µl to 5 µl of DNA (resuspended in H_2O and not TE or other buffer) to 50 µl of *Agrobacterium* electrocompetent cells and mix.

[000102] 2. Transfer 20 µl of DNA/*Agrobacterium* mix to cuvette.

[000103] 3. Electroporate:

25µF, 400 Ω resistance, 2.5 volts (0.2cm cuvette) or 1.8 volts (0.1cm cuvette for BioRad electroporator. 330 µF, 4000 kΩ, low w, fast charge rate for BRL Electroporator.

[000104] 4. Add 1ml of LB and transfer to Eppendorf tube.

[000105] 5. Shake at 30°C for 2 hours.

[000106] 6. Centrifuge down cells (2 min. 14 krpm).

[000107] 7. Plate all onto LB + antibiotics (most *Agrobacterium* strains are naturally streptomycin resistant).

<u>Example 3 – Protocol for Production of Transgenic Hairy Roots on Soybean</u>

[000108] Seed Sterilization. Rinse the soybean seed with 70% ETOH for 2-5 min. Remove and add 20% Clorox and shake for 20-25 min. Rinse 3X with sterile water. Plate the seed, 5 seed per plate, onto ½ MSB5 + 2% sucrose + 0.2% gel (referred to as ½ MSB5). Place seed into chamber at 25C, 16/8 photoperiod for 5-7 day (depending on genotype) germination period. After 1 week seedlings can be placed into cold room for longer storage if necessary (not to exceed 2 weeks).

[000109] Agrobacterium Preparation. For Agrobacterium rhizogenes strain K599, take a small sample from frozen glycerol into 25-50 ml of NZYM media with 50 mg/L kanamycin in a 125-250 ml Erlenmyer flask. Place onto shaker at 28-30 °C for 16 - 20 hours. Pour sample into centrifuge tube and centrifuge the bacterium at 4000 rpm for 10 min. Pour off supernatant and re-suspend the pellet with an equal volume of liquid ½ MSB5 + 200 μM acetosyringone. Use pipette to re-suspend the pellet and homogenize the sample (remove all clumps). To determine O.D., prepare a 1:10 dilution by putting 900 μl ½ MSB5 into cuvette and add 100 μl of bacterial sample. Determine the $O.D._{660}$ and calculate the volume needed to adjust (dilute) OD to approximately 0.2 for inoculation. Check final O.D.

[000110] Explant Preparation and inoculation. Place a sterile filter paper onto plates of 1/2 MSB5. Cut soybean cotyledons just above the shoot apex and place onto plate. Lightly scar the cotyledon's abaxial surface (flat side, upper surface that reaches toward sun) with a scalpel blade. Cut each cotyledon transversely into 2-3 pieces (no smaller than 1 cm). Add approximately 10 ml of prepared bacterial solution to each plate and allow cotyledons to incubate for 1 hr. Remove the bacteria using a vacuum aspirator fitted with sterile pipette tip, ensure that there is no standing liquid. Orient all explants with abaxial surface up and wrap plates for a 3 day co-culture, 25°C in light (16/8 photoperiod).

[000111] Hairy root selection and maintenance. After 3 day co-culture, wash explants with liquid ½ MSB5 + 500 mg/L carbenicillin. Transfer the explants abaxial side up to selection media, ½ MSB5 supplemented with 500 mg/L carbenicillin and 200 mg/L kanamycin. Roots should develop in approximately 2-3 weeks. The roots will form primarily from the cut vascular bundles with other roots developing from the small cuts on cotyledon surface. Remove roots (>1cm in length) and place onto replica media with

transfers to fresh media every 2 weeks to prevent *Agrobacterium* overgrowth. After 6-8 weeks on selection the roots can be moved to media without kanamycin, however carbenicillin must remain in media for several months for continued suppression of *Agrobacterium*. At this stage roots can be used for testing RNAi for nematode control. Sterilized nematodes can be added and observed for RNAi affects.

Example 4 – Testing of RNAi for Plant Parasitic Nematode Control.

[000112] Various types of nematodes can be used in appropriate bioassays. For example, *Caenorhabditis elegans*, a bacterial feeding nematode, and plant parasitic nematodes can be used for bioassay purposes. Examples of plant parasitic nematodes include a migratory endo-parasite, *Pratylenchus scribneri* (lesion), and two sedentary endo-parasites, *Meloidogyne javanica* (root-knot) and *Heterodera schachtii* (cyst).

[000113] *C. elegans*: RNAi vectors can be tested through expression of the RNAi in *E. coli*. *C. elegans* are fed *E. coli* and assayed for their growth by measuring growth of nematodes, production of eggs and viability of offspring. Another approach is to inject dsRNA directly into living nematodes. Finally, soaking nematodes in a solution of *in vitro*-prepared RNAi can quickly establish efficacy of treatment.

[000114] *P. scribneri:* The *P. scribneri in vitro* feeding assay uses a corn root exudate (CRE) as a feeding stimulus and both the red dye Amaranth or potassium, arsenate as feeding indicators. Feeding is confirmed after seven days by the presence of red stained intestinal cells in live worms exposed to the Amaranth or death of worms exposed to arsenate. This bioassay is used to test soluble toxins or RNAi. *P. scribneri* has also been cultured on wild type roots of corn, rice and *Arabidopsis*, and on A. rhizogenes-induced hairy roots of sugar beet and tomato. *P. scribneri* is very valuable in evaluating transgenic hairy roots because of the non-specific feeding of these worms.

[000115] *M. javanica*: Nematode eggs are sterilized using bleach and are used to inoculate hairy roots expressing RNAi. Nematodes are assessed for their growth by measuring knots, egg masses or production of viable eggs. An alternative approach is to microinject dsRNA directly into root feeding sites or into living female nematodes.

[000116] *H. schachtii*: Cultures of this nematode were maintained on sugar beets. Nematodes eggs are sterilized using bleach and used to inoculate hairy roots

expressing RNAi. Nematodes can be assessed for their growth by measuring knots, egg masses or production of viable eggs.

Example 5 – Plant Expression Vectors for RNAi

[000117] Modular Binary Construct System (MBCS): An important aspect of the subject disclosure is the Modular Binary Construct System. The MBCS eases the burden of construct development by creating modular pieces of DNA that can be easily added, removed, or replaced with the use of low frequency cutting restriction enzymes (8-base cutters). These constructs are useful for delivery of a variety of genes to plant cells and is not limited to the delivery of RNAi genes. To develop this system, a series of six, 8-base cutter restriction enzyme sites was placed between the left and right Ti borders of a previously created kan^R/tet^R binary plasmid (Figure 1). The production of both kan^R and tet^R MCBS aids the testing of constructs using different strains of *Agrobacterium rhizogenes* in different plant species. In addition to the MBCS, a series of shuttle vectors were created that aid in the cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites (Figure 2). With six 8-base cutter sites, each site is, preferably, reserved for a particular function (Figures 3 and 4). Because of the close proximity of the *Pme* I and *Sgf* I sites to the left and right border of the binary vector, these sites are, preferably, reserved for gene tagging and enhancer trap experiments. The *Not* I site is, preferably, reserved for plant selectable markers (Figure 5). The Pac I site is reserved, preferably, for Plant Scorable Markers (Figure 6). The *Asc* I site is, preferably, reserved for RNAi experiments (Figures 7 and 8), while the *Sbf* I site is, preferably, reserved for anti-nematode proteins. The restriction sites that are denoted in the Figures are, preferably, reserved for the denoted insertions; however, the MCBS binary and shuttle vectors do not require the restriction sites to contain these suggested inserts.

[000118] Plant Selectable Markers for MBCS: To further develop the MBCS, a series of plant selectable markers were added to the MBCS (Figure 5). Plant selectable markers that were added to the MBCS include: pNOS/NPT-II/tNOS (kan^R), pNOS/Bar/tNOS ($basta^R$ for dicots), pUBI/Intron-Bar/tNOS ($basta^R$ for monocots), and pUBI/Intron-PMI/tNOS (mannitol $isomerase^R$).

[000119] Reporter Genes for MBCS: Four exemplary reporter genes are used in the MBCS are provided in Figure 6 and Appendix 2. GUS, a nuclear localized GUS, GEP, and the anthocyanin transcriptional activator *papIC* genes into the MBCS.

[000120] Promoters for MBCS: We cloned several useful constitutive and nematode-inducible promoters (Figures 6, 7 and Appendix 2). Constitutive promoters include the SuperUbiquitin promoter from pine (pSU) and two promoter regions from the Strawberry Banding Vein virus ($pSBV_1$ and $pSBV_2$). Seven nematode-inducible promoters from *Arabidopsis* were also been cloned.

[000121] The following Scorable marker clones have been constructed and placed in the MBCS, NPT-II binary vector (pNOS/NPT-II/tNOS):

Intron/GUS/tNos	Intron/NLS-GUS/tNOS	Intron/GFP/tNOS
pSU/Intron/GUS/tNOS	pSU/Intron/NLS-GUS/tNOS	pSU/Intron/GFP/tNOS
$pSBV_1$/Intron/GUS/tNOS	$pSBV_1$/Intron/NLS-GUS/tNOS	$pSBV_1$/Intron/GFP/tNOS
$pSBV_2$/Intron/GUS/tNOS	$pSBV_2$/Intron/NLS-GUS/tNOS	$pSBV_2$/Intron/GFP/tNOS
pKT/Intron/GFP/tNOS		
pKA/Intron/GFP/tNOS		

Example 6 – Control of Plant parasitic nematodes using RNAi *in planta*

[000122] Production of RNAi Vector. The RNAi shuttle vector to be used is adapted from the Modular Binary Construct System (MBCS - See Example 5). RNAi shuttle vectors preferably comprise a promoter, intron, antisense RNAi, stuffer fragment, sense RNAi, and terminator (See Figures 7 and 8 and Appendix 2 for more details). The plant promoter can be constitutive, tissue-specific or nematode-inducible. The intron is necessary to eliminate expression in *Agrobacterium.*

[000123] The anti-sense and sense RNAi molecules comprise nematode-specific sequences and are disclosed herein. These genes are associated with pathogenesis, growth, or other cellular function in nematodes. An exemplary group of RNAi sequences for use in plant/nematode control may be based upon:

[000124] 1. Genes specific for nematode esophageal gland cells.

[000125] 2. Genes specific for plant parasitic nematodes but not other free living nematodes.

[000126] 3. Genes common to all plant parasitic nematodes.

[000127] 4. Genes common to all nematodes (nematode-specific).

[000128] 5. Genes specific for important tissues or cell types.

[000129] 6. Genes from large gene families.

[000130] 7. Genes involved in nematode signal transduction or other cellular pathways.

[000131] Appropriate RNAi constructs allow for the formation of dsRNA molecules (the sense and antisense strands join to form the dsRNA). The terminator sequence adds a poly-A tail for transcriptional termination. The RNAi shuttle vector can then be subcloned into the MBCS and transformed into *Agrobacterium rhizogenes*.

[000132] Plant Transformation with RNAi Vectors. An exemplary transformation system for generating hairy roots using *Agrobacterium rhizogenes* is provided below. The RNAi vector once introduced into the MBCS can subsequently (as a binary vector) be transformed in *A. rhizogenes* using, for example, the electroporation protocol of Example 2. Once the A. rhizogenes is confirmed to contain the plasmid, it is then used in generating hairy roots (See Example 3). Using this protocol transgenic hairy roots expressing RNAi are isolated, cultured and tested.

[000133] Testing of RNAi Vector for Nematode or Plant Pathogen Resistance. RNAi expressing hairy roots can be inoculated with sterilized nematodes. Infested hairy roots can be observed and the effect on nematodes determined. An alternative approach involves the microinjection of RNAi directly into root feeding sites (giant-cells for root-knot nematode, and syncytia for cyst nematodes) or into living female nematodes.

Example 7 – Insertion of Genes Into Plants

[000134] One aspect of the subject invention is the transformation of plants with genes encoding proteins of the present invention. Transformation of plants as described herein can be used to improve the resistance of these plants to attack by the target pest.

[000135] Genes, polynucleotides, and/or RNAi molecules as disclosed or suggested herein can be inserted into plant cells using a variety of techniques which are

well known in the art. For example, a large number of cloning vectors, for example, pBR322, pUC series, M13mp series, pACYC184, pMON, *etc.*, are available for preparation for the insertion of foreign genes into higher plants via injection, biolistics (microparticle bombardment), *Agrobacterium tumefaciens,* or *Agrobacterium rhizogenes*-mediated transformation, or electroporation as well as other possible methods. Once the inserted DNA has been integrated into the genome, the genetically modified-cell(s) can be screened via a vector carried-selectable marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G418, bleomycin, hygromycin, chloramphenicol, or bialophos, *inter alia.* The transformed cell will be regenerated into a morphologically normal plant. The transgene(s) in the transgenic plant is relatively stable and can be inherited by progeny plants.

[000136] If a transformation event involves a germ line cell, then the inserted DNA an corresponding phenotypic trait(s) will be transmitted to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

[000137] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

We claim:

1. An RNAi molecule, optionally comprising a linker, wherein at least one strand of said RNAi is encoded by a DNA sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 139.

2. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 1.

3. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 2.

4. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 3.

5. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 4.

6. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 5.

7. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 6.

8. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 7.

9. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 8.

10. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 9.

11. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 10.

12. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 11.

13. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 12.

14. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 13.

15. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 14.

16. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 15.

17. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 16.

18. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 17.

19. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 18.

20. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 19.

21. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 20.

22. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 21.

23. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 22.

24. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 23.

25. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 24.

26. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 25.

27. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 26.

28. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 27.

29. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 28.

30. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 29.

31. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 30.

32. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 31.

33. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 32.

34. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 33.

35. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 34.

36. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 35.

37. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 36.

38. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 37.

39. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 38.

40. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 39.

41. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 40.

42. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 41.

43. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 42.

44. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 43.

45. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 44.

46. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 45.

47. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 46.

48. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 47.

49. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 48.

50. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 49.

51. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 50.

52. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 51.

53. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 52.

54. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 53.

55. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 54.

56. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 55.

57. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 56.

58. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 57.

59. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 58.

60. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 59.

61. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 60.

62. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 61.

63. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 62.

64. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 63.

65. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 64.

66. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 65.

67. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 66.

68. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 67.

69. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 68.

70. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 69.

71. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 70.

72. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 71.

73. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 72.

74. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 73.

75. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 74.

76. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 75.

77. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 76.

78. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 77.

79. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 78.

80. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 79.

81. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 80.

82. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 81.

83. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 82.

84. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 83.

85. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 84.

86. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 85.

87. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 86.

88. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 87.

89. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 88.

90. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 89.

91. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 90.

92. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 91.

93. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 92.

94. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 93.

95. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 94.

96. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 95.

97. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 96.

98. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 97.

99. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 98.

100. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 99.

101. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 100.

102. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 101.

103. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 102.

104. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 103.

105. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 104.

106. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 105.

107. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 106.

108. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 107.

109. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 108.

110. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 109.

111. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 110.

112. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 111.

113. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 112.

114. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 113.

115. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 114.

116. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 115.

117. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 116.

118. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 117.

119. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 118.

120. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 119.

121. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 120.

122. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 121.

123. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 122.

124. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 123.

125. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 124.

126. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 125.

127. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 126.

128. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 127.

129. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 128.

130. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 129.

131. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 130.

132. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 131.

133. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 132.

134. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 133.

135. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 134.

136. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 135.

137. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 136.

138. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 137.

139. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 138.

140. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 139.

141. A transgenic plant or transgenic plant tissue comprising an RNAi molecule according to any of the preceding claims.

142. A method of disrupting cellular processes in a nematode comprising the steps of:

(a) providing a composition comprising a compound according to any of the preceding claims; and

(b) contacting a nematode with said composition.

143. An isolated promoter comprising the following nucleotide sequence:

```
          aacagcccaagataacagaaaagtcaaaggtgttcgaaa
gaccacttgtgactaaggatcatttcatccataattatctggtagca
cagactcatgataactgcgaggaacacaagttctttacagtcgattc
aaagacactttctctttacggtttcattgaaggagccgacccagaat
atgtcagagaagcttttcactgtgggttaatttcattaatctatcca
ggtgaaaacctcaaggagatctctcttctcccaaaagacctctacag
ggcaatcaaaaactacagaaccagagtttgtagtgcacagagtagac
caatctacctgagaatcacgagtaccttcctagagtgggaaaatgat
gacatccttattccataccactggattgaggtaggactatccaatgg
aaaaattccatggacaagtcatataagaagaccgcaacagtcgagt
atcttccagagataactgcactcagacctaaaaggataaaagcagta
tataatcagtgtactaagatcttcgcagattcaaagaagaagcttaa
ctatgctgatgacaagataattctaataagcaattattcagaattaa
tcaaggagaaagaattaataactctttcagaatatgaagcccgcttt
acaagtggccagctagctatcactgaaaagacagcaagacaatggtg
tctcgatgcaccagaaccacatctttgcagcagatgtgaagcagcca
gagtggtccacaagacgcactcagaaaaggcatcttctaccgacaca
gaaaaagacaaccacagctcatcatccaacatgtagactgtcgttat
gcgtcggctgaagataagactgaccccaggccagcactaaagaagaa
ataatgcaagtggtcctagctccactttagctttaataattatgttt
cattattattctctgcttttgctctctatataaagagcttgtatttt
catttgaaggcagaggcgaacacacacacagaacctccctgcttaca
aaccatgtattgtagctaaacctcttaggag .
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144. An isolated promoter comprising the following nucleotide sequence:

tggtggggacaatggatccggtctgcgtagcaacaaggctg
aaaaagattaaacagaaacctgtgatcattagcgttggaccaccacc
aaaacctcctgagccaccaaagcctccagagcctgaaaaccaaagc
ctccaccagcacctgaaccaccaaagcatgtatgcaagccaccttac
tgcaacagttgtgatgttgtgtctgttactacctatgaaagtggaag
cggctgcaccattctttgagtcatatatcgcgtaccatagccttcat
gttaagtcctgtatttagccaatactaattcatcatgttctcatgct
tttttgtttatttctttttctcaaatatgaatctctgttgtttgtcc
ctcccctgtttataattagtcgcttctttgacacaagaagtctcatg
agttcatgctaaagaaaataaaagttcaaattaaaacaccaaatgtt
tgattaatttccataaacctgtgaagcagaaagttagtcatgttgac
ctgaacagagcttaggaagtccttgaaggacatatcttcaagtgcta
ttgggtcgtagcactcttaggcccattaacttcattgagcccattaa
attatgcaaaacaagaaatgagacatatggaaacattagggttctta
caggaaaaaatagggaaaaagcagggacaactaaacaaaaattcagaa
acaagaggcaagtggacgaccacggcgtaagatcaacatgtggtgat
gtgcatgagaccaagaccatttttctcgttcttcaacgcacacttg
gtcttttcttatgtttgttgcatttctttattaggcagaccctctct
ctttttaataggatagtaaaaaatatatgatttatttttgttgaaa
cattttgagttaaaacctaaacttatagtaagcatttgtagagtgaa
tttcctatacgacatctatcaacatgacctctaaccaaaaaaatatt
gatgaaactactttaagtagtaaaacctaaagcaattaaaatttcct
ttaaattagtagtttgtgtaaattaattgacatgattgcgtcgaaag
aaatcaaaacagttatatcgtgaacttaggagaatgttttatatcgt
gtttcaacacatgattgctagcatatgtgtaggtgtcgtagacgtta
cataacaatcatcactcgtaaatatcaaagtggtttctgagagaaac
aaagggttatgattttcccaactgcactagttgtgtattgtttcttt
cacacgtatgcttctgagttctgcccaaagtggaaattaaagcagag
ttgggagagatcataatttattagggttcgttatgctcaagtcatga
cgtaaaatgaaaatttgtttttattctttcaccaacacaaagaatag
ctagttatctctttttttatatataacaattcatgaagttgatcagc
tttatacacatcatccaatcgaattgctaatctagagatggaaatat
caggatagagccaataagatatcaaatccaatggacccattttctcc
atgtgctaattcatacaatctgtttttgtctgctttatttgatgatg
atgctgagcgtttttaagtgtgaactaagatctagctaaccaaacaa
aagatggtctcttctgtctttgtcgtataagagcaagagagtggttt
gattcaattttaaaattctaaataaaactccaaccgtgaatccagc
catgaaactctttttagaaaatcctttttataacaaataattcctc
tgcttcttcttcttcttcgtttatttcacctttttggtttctttag
ctcagaaaaagcccattctttttttctattcttgtttattttaatca
tactgtgcgtttctacaaagtttgttcctttcttcttcaactctctc
actcacagtcacagagatctgtttcttttcttttttgctttcactc
ttctcttccagt.

145. An isolated promoter comprising the following nucleotide sequence:

agcaaagcaagaacaccagagaagaagaaaagcactacaga
gaaaaatgtgagcttaagcgctctccaacaacacttctctgggagtc
taaaggatgctgcaaaaagccttggtggtgagacttccgcatatttc
caagcatgggtttattttgttagcacacaaactatctgaccctcga
cttggattttcttctgcagtttgtccaactacattgaaacggatatg
caggcaacatgggatcatgaggtggccatctcgtaagattaacaaag
tgaacaggtcactaaggaaaatacagacggtactggactcggtccaa
ggtgtagaaggaggactaaagttcgactcagcaactggcgaattcat
tgcagttagaccttttattcaagaaattgatacccaaaagggtctgt
cgtctcttgataatgatgcacatgcaagaagaagtcaggaggatatg
cctgacgatacttcattcaagctccaggaagctaaatctgtcgacaa
tgccattaagttagaggaggatacaaccatgaatcaagcaagaccag
gtaagaacttctctatccataaaccatagatggagcgattagaatct

taatccattttcagttttgcaggatcattcatggaggttaatgcta
gtggtcagccatgggcttggatggccaaagagtctggcttgaatggc
agtgaaggaataaagagcgtttgcaacttaagctctgtggaaatttc
agatggaatggatccaacaatccgatgcagtggcagtattgttgaac
ctaaccaatccatgtcatgcagcatatcagattcatcaaatggctca
ggcgcagttctgcgtggaagctcatctacttccatggaagattggaa
ccaaatgagaacccacaacagtaatagcagcgagagtggatcaacaa
cgctgatcgtaaaggccagttatagagaagacactgtacgtttcaag
ttcgagccatcagttgggtgtcctcagctctacaaagaagttggaaa
acgttttaaactgcaggacgggtcgtttcagctgaagtacttggatg
atgaagaagaatgggtgatgctggttacagattctgatctccaagaa
tgtttggagatattacatggtatgggaaaacactcggtgaagtttct
cgttcgtgatttgtctgcccctctaggtagttctggtggcagtaatg
gttatcttggaacaggcttatgacgtcgtaagacatagacacacaca
gttatgtattcccagtgaaagaatgttgtttatttctctagatatta
gtatgcttataaataggcatgaaggagaaagacaatttggtatagt
ggagttcagcagaaaatgtatatgtttttcgttttatatgaatcag
agaataaaagttggatgttatatctacgttgctaatgttgtacctgc
tcacccatctttcatataagaaaagagaacacttttagttatccctg
tgatgcagaatcgtattctttgttatctctccattcctgtggaaacc
aacaaagtcaactaaatttcggtttaattggttggtttttaagtcaa
cgaggacttgattttagttgggcttgggcctataattgtgttcatca
ttgggtttttccccttatcagtttaacgtccatatccatatcttt
ttcttttttaacggcaaagttcatatccatatcttatgatgtgcct
aaaagagggagaagatgcgaagacagaattttcatatttgaaagggt
tcgatatcgatattgggaaacgaatcaaggtcaaaaaactcagtcta
atagttgaaatttaaaaattttattaattcaatccgattggtttcgt
tttgttatggttcggttctatatcatcaaaccaatcggtttggtcct
aaagataattataaatattcaccaacaccagtgttaaacacatatca
acaaacctaaagttagataaacaaagaga.

146. An isolated promoter comprising the following nucleotide sequence:

aattggcactcttcttctgctgggttccaaaagaaacgaat
caatatgtgcaacaagaagagctccagaagcagtcatcttctaaaat
cttaatctaacaacagctcaagaagaaaaaattccatagctagaga
gaacacaaagtcacaagacgacgtcgtagaggcacaaagtcaaacct
gaatggcttaagccgaactgagtggtttgactagaccatcatcaga
aaagtctccaagacggtagtcggatgttagatcgctcaagtaatttt
tggttttgttggtctcacgttttcagctgcccatttgatttcagttt
gggcttttccttatctctaaaggcccaatttcatttaggtttagttt
atttgatcattatccttactataaaggcttcgcctttcgagaaattt
agggtttcttctgtctgtctcgtcactcaggtttgtgcctcaacgac
tgcttcacttctagcttgattcttcttcttcgtttatatgtatactg
tacattagattattcttgtttctcgagcttctgctatagattttgat
tctttttttgttgtctttgtttcgtttccaggatcagatcttagct
aaattgagacaagctcaaaatgaggtacttgacgcatctcttacatt
cactgtttaattagagaacaatacgtctctgaatcgtgattcagaga
cgtattgttcttctgtcatatgcaataagtttaattagagaacaata
cgtctctgaatcgtgattgtttttggatgtgcgttattgatagctt
tatgatgttaatagtctaggattgacacgaagttgttctgcagtttt
gcataaatgctctttactaaggcctctaaatttggatgacaaatcta
aatcttgcctcataaaaatttaggtgtattaagataagattattttg
tatggtagtgtctataatgtgggttgttcatgttgaggttgtcaatg
ttgtgtatttttgtttgtttagttaatttgcttaactctgttctttg
tgggttaatacagtaagcttcagagtgaggccgttcgtgaagccatc
actactatcacagggaaatccgaggcaaagaaacgtaactttgtcga
gactattgagctccagatcggtctgaagaactatgaccctcaaaagg
acaagcgtttcagtggatctgtcaagttaccacatatcccccgtcct
aaaatgaagatctgcatgctcggagatgcccagcatgttgaagaggt
gatatatcttttcatggaaattgatcattttgtgctctgtttcttgt
ataatggttttgtgctcatttcatttggtggctctattagtttcatt
tgatgttgtatatgtcttctgaatgtagatgcatgatgttttcggaa
tttggtcattgtttatttaggcttcatttcttgcataattaaatatt
tgcttatttcatcttgtatcttttcgtaggctgagaagatggggttg
gaaaacatggatgttgagtctctaaaaaagcttaacaagaacaagaa
actcgtcaagaagcttgcaaagaaataccatgctttcttggcctctg
agtctgtcattaagcagattcctcgtcttcttggtcctggtcttaac
aaggcaggcaagttctggctacagctaatattccattgttcttcttt
acatccgttttgattttggataggttttagtagtctatttcttttgt
caatgtctttttgatacaatgccaatcctttatcctgtgagattatg
cttctttgatgattcttaagtaacattcctttgctttactttacaca
ggaaaattcccaactcttgtgagccaccaggaatccttggagtcaaa
ggtgaatgaaacaaaggcaacagtgaagttccagctgaagaaggttc
tgtgcatgggagttgcagttggtaacctttt.

147. An isolated promoter comprising the following nucleotide sequence:

tggcaaactgagatataagagggaaggtgatttcatgcaa
attttttttttatttttttttgaatgaatgcaaaatttattcaaaaa
aaaaaaacctgggctacatcaagtacttcatttctgagttttgaaa
aatctaaagacaacaaaagactttacaatttaataaaaaaataataa
aaatactttatcactctcaacgaaattgttgatttaataacgtatct
cttggtaaaacagcgttttatttgacgaaattgttataaatgaataa
aatgataatagaaactagtgtggtacgtaaaatacctctcatttggc
aaaataacggttatgtatcatgagtattgcatacgacagcgtgctta
aatagtgtgctttcaggagaaaatatataccaagttatttgctgaaa
ttaccacgcaaatctgaggttcgaatggcaaaataaaaaaccaatgt
catttccttaatgtattaaggtcatttaaataaaattgtacactttt
ttcacctgtaagcgttccaaagtgtagaatggataactagaagggtc
aaaggtataatattaataagcgaactcacttttgcccaagtgattt
cacttcttacatttgcttgatatagttacccaaaagtgtatatatat
tcccttatacaattgttctatttctggattataaggggaataagaa
aaaagaaaagagagagtatataataatacttttataaagtgatgtta
gattctaatttgtaacgaaaagttcaaagtgaaagaaaaaacgaaaa
agtttttctgttttgttttatatctatagccaagaaagtttctcaga
tttacaagaagttaactgagaaaaacaaaaaaaaaacttatgaagca
tgaaagactaattaacgaggtgattaatttgagacaaattaaacat
cgaattaaaagtaacatttggagggtttatatgttatatatgtgaca
tgataagtccgattcatgactaatgtatatctggaatctaacatgga
agaatagagaacgaagcagagccaaggtcaacttgccagacacgaat
caacagattgtgaatgagaccaaatcaatggtcataaaccggttggg
tttaaaccggcaagtcatccttggctcaattccattcgttattcctt
catgcaagaccctctgatacaaccaaagactcccattacaatattct
ttcgatcacgagctacttattttcaaatgtgttacctctttcgtgac
tcttgtgttgtgtggtaaagcctagtcgagatgtgtcggtatatata
ggcatacatatacaaatgcgacaaaataagtatattatattgtttaa
tttctatattccatttctatatgcatggctgggattttgaccaaaa
ccctaattcaagaatagaatccaaaagatgggatcaaagaatataat
ctaatgggctgaccacattttccgatttaattcgcatagttaatatt
ctttccactactttatgccgcagaaatttgtaattaagtaagacaaa
gaaatacagatataagatggtcgtagaaaccagtagaggaatttcat
ttttcgtggataagtggaatattaataagagaatggtctttactctt
tacagtgggaaatgggaatagtagcccattataatttcatcagattc
tatatatgcatgtttgtataagctaaaataaatacgtttaagcattc
ttcaaaaaaatttacaagttctagagactctcttaacgtcggcaatt
tatattctactttacatgacactttcaggaaaagaaaactatactca
ctagcagatcattaaatttttcttttttctttttttgaatgaaccttag
ttgtggttttattttgttagctagaaacttcagtgtttttttttcc
gccaatggtagtgctttgatgatggtccgg .

148. An isolated promoter comprising the following nucleotide sequence:

caatcaaggtaacgaaggaggatcagcgaaaggatgggcta
tatttggagtttttcctgcgtgtaagtaatgctttgtgatcttcca
tgcggacatataactgaagaataaactcaactcattgtgttctggtg
tgtttcttctgatcagattcctcgttgcatctgcacttttctgctgt
gggggctttatttataaaacaagagtagagcgtgtggtaatcttcat
atctttctacaattccacttccattctctaattattctctcacgtga
tatacacacactcaatcactgatgtactcgtatggatgcagcgtgga
actgatgcattgccggggatgtcacttctatcgggcttactagaaac
tgtaagtattacaagaaaactcaaaaggattccatttatgcaaaatc
taagagaaagctcactgtggtctttggttacaatttatggatctctc
aagagacaaatgctatgtaagctaattgattttggtcttgataaaca
ggtgagtggaagtggacaaagctactcaagaactgaagacatcaaca
atgcttttgccaatgaagtctcatgggaccgctcttccgcatcttct
actcaagcgacaacaacacagagaccaagtgaaagaacatatggtgc
gatctaatttgtcaagtgcctcacaagaggtactgtttcaagccat
ggtatggcacgcttgtgatctgcgatttctggattttgctttgtatg
tttattttctaccttctagaaagaggtcaaaaagttaatagcttcac
cgtgagaatgttgttttcaccagattcatgtgctatgatagaaaaag
acaaagcaaacaagagttctttctttgcttaggttacaagaacaaga
gtatcgttataaagtcaacaaagattgaaacatatttttgtcaaggg
agtggttagaatctcttcctactctcttgcctttctcactaagacaa
aaaaaagacttggactttgtctaaggttttgtggatattattaacca
agtccttttgcaaaaagtaatattgtttttttcgcattcctcttttag
aatttagtttaatctaggctttatattggttattactttcttgaaaa
atgatctgtttattctattcatacttggttacctcgctttttatctt
acttctacaaaaggattatcagtgaaagttagtctcttactctcacc
ttccgaaaataaaacaaaaatatcgatacttctagatcaaaccaagt
tgattaaaacatccctattccctacgattctgatcttgagatatatt
atcatgttaagatctaaattgacaagaaaactgatttttcatttcta
gtaggaaaaataattactattagtgatcatgattgtcgaccgtaaga
ggtggtttagttactctccatctttctttgaagaagtcagaaagtca
gaaattatatcaaattaaacatcaatattgaacacatatatctgtat
ggttttatgtttagaaaattccaatatttatatattcctagggaaaa
agaagcttattcttcaaattattgttatgagtcgttaaaatatggat
aaaaatataaagtctaaatattaaaaactcagtttgctttgcttta
cctctccaagtctccaaagtcaaattaattttagttaattaaaccaa
aaaaggtttattagtcaaacttagcatgcaatgctgggtaccaaacc
caagcattagtctcttttaatcttctttttctccaataagtttttac
aatttttaattgtttgcatttcccttgattatttatcttcatcccaa
tttagctaataccaactccgtttcttattcttccaagtcttttccta
taaatacgttcttcttcccctcttatttcatatcactcaccacaaag
tcttctcatttcctcat .

149. An isolated promoter comprising the following nucleotide sequence:

atgttgtgagtgaaggagaagaagagggaaacaaaggtatt
tatttgtagcgagttttgttttgtgacgcggttttgtctgtgttcaa
tgttgacgaaacgagtgagagagtgtctgattattaaagaaaaccct
aattaagtcagacccgccggttataaaaatagtcaaaaagtaggaaa
acgcgtgtgtgagtgagacagagacagcccattgtttgctttatggg
cttataagcgagacgtgttaattgggctttttcctttatggccgaaa
acaaaagaaacgtcgcctgagagattcgaactctcgcgggcagagcc
catgtacttagcaggcacacgccttaaccactcggccaaagcgactt
gttgctatgagttagacaaaatcattaaaattctctattatgatttc
tcatagtgtgtgtgtatattgtggatctactaaaaattctttgttat
tattactttattttgtgaattagtttgatataggtaagtacaaagtt
aactttattatttactcaaaatttatcagattaactgattttatatt
gtttcctttggtatatagacgtactatagttttagaaaaaccataa
gattcctttatatttcatagagtgaagagatgagatgagatcttggc
tggagaagaaataagtttccacgaggaggactctttttttttggtga
agacgaggaggaggactcttggttgatccagtctttacgttagacat
cgacccctacatttatttgcctttctctatcaacatggcaggtaaaa
atcttcattcaaccgaaccaaccaaagtctcttcccaataatattca
agcaccatcctttgggaaactcatacatactacagtctacactcttt
cattttctttcaacgctcaacttaacaaatgatatagtctagttgtc
aattatatgttttaattagtgttttcacatcaaattctggtttgata
tttgatgactatttcggaaacatctcaatgtcccgcaaatacaatc
gtctatcatatataatcccgtacgttgtattcttatagatagaataa
tatggcgtgatctttataatataacatatagaatcgtgtagatttat
tttattttattttatatatcgcataaattgcaaaatacttatatat
gtttgttatatatgatacccatttttatagttacttaaaaaaagttaa
gcgataatatatatatatcaacttttttataacaaaaaagtataacac
atggtaaagaaaaataaaaatgaagacatggtgtgacacgaaaatgg
cactaaatatacatatataatagatagctacaatatcccatcataca
cactttttaattgactaatacataacttacacactttttaattga
ctaattcataacttttttatcattgtcaacatgcaaattcatatttcc
gttgaactattattcttatttgttttaaaagaagggcttcctggt
aataaaaatatgatttccaaatgacgttagagcaaaaaaaaaaaag
gttgtctggtctggtaaaatgaaaaagcaaagcgtcttggtatagaa
aagtaatatactgcctcctaatttcttcgtccttctaccgaagaatc
tctccactcttgccctctttcgaaaccctaaaccagaagcaccagat
tttttcaacttttcccagagaacaatagaaaacccaacttgtgctc
tctagggtttctttattccttctcatctttggatttcttgggtca
tcattttggaagcttacccaccagcgaaaaaattataacttccatcg
attcctggcttctctctctcgctctctctgcatgtgctaaatcgccg
gactgatcctcactgtcacctctgtt .

150. An isolated promoter comprising the following nucleotide sequence:

gattaggggtttgagttgtcactggaaagaggtttgattgt
gagtgatgatggagagattatgaaggagtttgtgtgtatttatagag
gagttagggtttgaggtttgatgagaagtaggtttgaagaagtttt
gttgttgcaacttatttagagttacttgttccacaaccacaagtaag
attggtcacttctaagttctaactagaaacaaccatgacacatggag
atttcagctaacctagtttaatgtatatgtattatattttatttaaa
tattataaaataaaataaatttcacaaataaaagaactacaaaaaa
gtgagaaaaataatttgataaacaaatttagaaaattagtatatcaa
taaataaatttataatccgatggttttgccttttggtttggcctttg
tttgaacttcgatgagtgactatgtatagcgaaaacaattcggtttg
tttttggtttaattttaaaaaatacaagcgacaatatctgatgagaa
taggtgaaaagcaaataatatcagtttaattggaaatatttactttt
ttacaactaatattttgtttggtcaaccaacaaatagatttaattaa
ttatggtttatgagcttttatttgttgcgacagtatatatatgttaa
aatagtgatattgcatggcggaaggtccggaagcaacacatatctcc
ttttaatttttttttaacaagaataacatgttaattttttttga
aattaataaagaatacatatttctaattttgcgtcagatagatgat
taaagagtgtgtgtttttttaacaaacaaggaatacattatacata
tttcatatttctctcgacattgtttgtttttttaaaaaatagattaa
agagtctacgaagctaagtagctaacgaagacttgaaatgagaagaa
gacgagaatcttttaatatttttttgttaagcgataatattttgaaaa
ttaataaatatagattaaggaaataacaataacgcagatatcggtaa
gtcatagaaaaaaagaaacaacacaaacttacataaacatgtttcct
aatttgtaatggagtaaaattccttcttttttttttttttgattt
ggattccaattagtaaagaactcaatgactataaataacctttaacc
ctctcattatttcttactatcaattgattaagctctcgttcctaaga
aagcaatagacgaacaagaacccatcgaagaacacaaatctctcttt
gaagttgtcgataatgttagtacaccgttacttcgtccaagactttt
ttgccgttccgtttcttacaaaacaaggatttggttaccattacttt
tgtcgtaactccttttacatgtacgtcaaaaagtggttcctcgctc
cggcttgaagaaacgaccttcttacccacaaaaagcttattttaaac
cgtctaaaaccggaaaatctcaatctaaaccggatacggttcatgag
aaaccgattcaaacaccgagtgaagaagtagaatttttgatggttc
cgtcacaatgtgtgctgctccttcgccaagacatgtaccgattccga
tattttgtggtgtaaagatgatcaaagagtcttcaaagctaagcacg
acttgaatgagaagaagaagaccaattactcaattagattttgtttt
gtggagcaattattgtctatttatctttgttttagcaaataatctg
tatccactaatcttcacagtacttgactaacaagaagtaaagagttt
tcttatttccaattgtttttaatctgatactttttcataatttta
caatgtttgatgaaaaaaaacattcaaacctaaatttctttttttg
gtatgaattcaaacctgaattacttttgacgaggacccgacggtata
aatagggtgatctcccaacaaacaaaaaggt.

151. A transgenic plant or transgenic plant tissue comprising an isolated promoter according to any of claims 143 through 150.

APPENDIX 1

SEQ ID NO:	INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
1, 2, 3	2293133	glyceraldehyde-3-pho sphate-dehydrogenase
4, 5, 6, 7	7143495	Histone H4
8 & 9	7143515	ATP dependent RNA helicase, mRNA sequence
10, 11, 12, 13	7143527	nematode specific
14 & 15	7143602	protein serine-threonine phosphatase 1, catalytic subunit
16 & 17	7143612	40S ribosomal protein S4
18	7143666	cytochrome p450
19, 20, 21, 22	7143675	Neuroendocrine protein 7B2
23, 24, 25	7143839	nematode specific
26	7143863	40S ribosomal protein S17
27 & 28	7144016	vacuolar ATP synthase subunit G
29	7144025	malate dehydrogenase
30 & 31	7144060	J2 pcDNAII Globodera rostochiensis cDNA similar to Bystin, mRNA sequence
32 & 33	7144225	similar to arginine kinase
34	7144354	pyrroline-5-carboxyla te reductase

SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
35, 36, 37, 38	C10	ribosomal protein L18a
39, 40, 41, 42, 43	C118	ribosomal protein S11
44 & 45	C122	ribosomal protein L16/L10E
46 & 47	C127	FMRFamide-related neuropeptide precursor
48	C129	ADP-ribosylation factor 1
49	C130	ribosomal protein L11
50	C137	nematode specific; conserved in *C.elegans*
51 & 52	C138	ribosomal protein L7
53	C145	ADP/ATP translocase
54 & 55	C148	troponin
56 & 57	C154	calponin
58	C16	translation elongation factor EF1A
59 & 60	C18	40S ribosomal protein S16
61	C27	ubiquitin
62 & 63	C46	nematode specific
64, 65, 66	C48	ribosomal protein S3AE
67	C59	40S ribosomal protein S5/S7

SEQ ID NO:	<u>APPENDIX 1 (cont.)</u> INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
68	C8	glyceraldehyde 3-phosphate dehydrogenase
69 & 70	C82	60S ribosomal protein 130/L7E
71	C90	glyceraldehyde 3-phosphate dehydrogenase
72	C135	nematode specific
73& 74	C206	predicted troponin
75	C227	cytochrome P450
76	C238	vacuolar ATP synthase subunit G
77	C246	40S ribosomal protein S4
78	C308	FMRFamide-like neuropeptide precursor
79	C342	ubiquitin
80 & 81	C344	nematode specific; conserved in *C.elegans*
82, 83, 84, 85	C370	40S ribosomal protein S5/S7
86	C426	nematode specific
87	C458	histone H4
88 & 89	C481	ribosomal protein L30E
90 & 91	C556	nematode specific; conserved in *C.elegans*

SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
92	C628	ribosomal protein S17E
93 & 94	C665	malate dehydrogenase
95 & 96	C669	malate dehydrogenase
97	C694	ribosomal protein S3AE
98 & 99	C709	ADP/ATP translocase
100 & 101	C714	ADP-ribosylation factor 1
102	C721	calponin
103 & 104	C726	ribosomal protein L11
105	C736	nematode specific
106 & 107	C773	troponin
108	C834	nematode specific
109	C860	bystin
110 & 111	C863	troponin
112 & 113	C883	translation elongation factor eEF-1A
116	C888	40S ribosomal protein S16
117	C898	glyceraldehyde 3-phosphate dehydrogenase
118 & 119	C935	peptidyl-glycine alpha-amidating monooxygenase
120 & 121	C937	calponin
122 & 123	C942	peptidyl-glycine alpha-amidating monooxygenase

APPENDIX 1 (cont.)

SEQ ID NO:	INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
124	C954	arginine kinase
125, 126, 127	C969	calponin
128 & 129	7235653	ribosomal protein L18A
130	8005381	neuroendocrine protein
131	7235496	pyrroline-5-carboxylate reductase
132 & 133	7275710	protein phosphatase pp1-beta catalytic subunit
134	7923685	nematode specific
135	7641370	40S ribosomal protein S11
136 & 137	7923404	nematode specific
138	7797811	ATP-dependent RNA helicase
139	7143613	predicted phospholipase D

Appendix 2:

Exemplary genes used for RNAi vectors.

Promoters:

Constitutive:

Super Ubiquitin from Pine

CCCGGGAAAACCCCT CACAAATACATA AAAAAAATTCTT TATTTAATTATC AAACTCTCCACT ACCTT
TCCCACCAACCGTTA CAATCCTGAATG TTGGAAAAAACT AACTACATTGAT ATAAAAAAACTA CATTA
CTTCCTAAATCATAT CAAAATTGTATA AATATATCCACT CAAAGGAGTCTA GAAGATCCACTT GGACA
AATTGCCCATAGTTG GAAAGATGTTCA CCAAGTCAACAA GATTTATCAATG GAAAAATCCATC TACCA
AACTTACTTTCAAGA AAATCCAAGGAT TATAGAGTAAAA AATCTATGTATT ATTAAGTCAAAA AGAAA
ACCAAAGTGAACAAA TATTGATGTACA AGTTTGAGAGGA TAAGACATTGGA ATCGTCTAACCA GGAGG
CGGAGGAATTCCCTA GACAGTTAAAAG TGGCCGGAATCC CGGTAAAAAAGA TTAAAATTTTTT TGTAG
AGGGAGTGCTTGAAT CATGTTTTTTAT GATGGAAATAGA TTCAGCACCATC AAAAACATTCAG GACAC
CTAAAATTTTGAAGT TTAACAAAAATA ACTTGGATCTAC AAAAATCCGTAT CGGATTTTCTCT AAATA
TAACTAGAATTTTCA TAACTTTCAAAG CAACTCCTCCCC TAACCGTAAAAC TTTTCCTACTTC ACCGT
TAATTACATTCCTTA AGAGTAGATAAA GAAATAAAGTAA ATAAAAGTATTC ACAAACCAACAA TTTAT
TTCTTTTATTTACTT AAAAAAACAAAA AGTTTATTTATT TTACTTAAATGG CATAATGACATA TCGGA
GATCCCTCGAACGAG AATCTTTTATCT CCCTGGTTTTGT ATTAAAAAGTAA TTTATTGTGGGG TCCAC
GCGGAGTTGGAATCC TACAGACGCGCT TTACATACGTCT CGAGAAGCGTGA CGGATGTGCGAC CGGAT
GACCCTGTATAACCC ACCGACACAGCC AGCGCACAGTAT ACACGTGTCATT TCTCTATTGGAA AATGT
CGTTGTTATCCCCGC TGGTACGCAACC ACCGATGGTGAC AGGTCGTCTGTT GTCGTGTCGCGT AGCGG
GAGAAGGGTCTCATC CAACGCTATTAA ATACTCGCCTTC ACCGCGTTACTT CTCATCTTTTCT CTTGC
GTTGTATAATCAGTG CGATATTCTCAG AGAGCTTTTCAT TCAA**CCCGGG**

Strawberry Banding Vein Virus 1

aagcttttcactgtgggttaatttcattaatctatccaggtgaaaacctcaaggaga
tctctcttctcccaaaagacctctacagggcaatcaaaaactacagaaccagagttt
gtagtgcacagagtagaccaatctacctgagaatcacgagtaccttcctagagtggg
aaaatgatgacatccttattccataccactggattgaggtaggactatccaatggaa
aaattccatgggacaagtcatataagaagaccgcaacagtcgagtatcttccagaga
taactgcactcagacctaaaggataaaagcagtatataatcagtgtactaagatct
tcgcagattcaaagaag**aagctt**

Strawberry Banding Vein Virus 2

Gtttaaacaacagcccaagataacagaaaagtcaaaggtgttcgaaagaccacttgt
gactaaggatcatttcatccataattatctggtagcacagactcatgataactgcga
ggaacacaagttctttacagtcgattcaaagacactttctctttacggtttcattga
aggagccgacccagaatatgtcagagaagcttttcactgtgggttaatttcattaat
ctatccaggtgaaaacctcaaggagatctctcttctcccaaaagacctctacagggc
aatcaaaaactacagaaccagagtttgtagtgcacagagtagaccaatctacctgag
aatcacgagtaccttcctagagtgggaaaatgatgacatccttattccataccactg
gattgaggtaggactatccaatggaaaaattccatgggacaagtcatataagaagac
cgcaacagtcgagtatcttccagagataactgcactcagacctaaaggataaaagc
agtatataatcagtgtactaagatcttcgcagattcaaagaagaagcttaactatgc
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gaagataagactgaccccaggccagcactaaagaagaaataatgcaagtggtcctag
ctccactttagctttaataattatgtttcattattattctctgcttttgctctctat
ataaagagcttgtattttcatttgaaggcagaggcgaacacacacacagaacctccc
tgcttacaaaccatgtattgtagctaaacctcttaggag**gatatc**

Nematode Inducible:

Trypsin Inhibitor from Arabidopsis (clone#6598343)

cccgggagcaaagcaagaacaccagagaagaagaaaagcactacagagaaaaatgtg
agcttaagcgctctccaacaacacttctctgggagtctaaaggatgctgcaaaaagc
cttggtggtgagacttccgcatatttccaagcatgggtttattttgttagcacaca
aactatctgaccctcgacttggatttcttctgcagtttgtccaactacattgaaac
ggatatgcaggcaacatgggatcatgaggtggccatctcgtaagattaacaaagtga
acaggtcactaaggaaaatacagacggtactggactcggtccaaggtgtagaaggag
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aaattgatacccaaaagggtctgtcgtctcttgataatgatgcacatgcaagaagaa
gtcaggaggatatgcctgacgatacttcattcaagctccaggaagctaaatctgtcg
acaatgccattaagttagaggaggatacaaccatgaatcaagcaagaccaggtaaga
acttctctatccataaaccatagatggagcgattagaatcttaatccattttcagtt
tttgcaggatcattcatggaggttaatgctagtggtcagccatgggcttggatggcc
aaagagtctggcttgaatggcagtgaaggaataaagagcgtttgcaacttaagctct
gtggaaatttcagatggaatggatccaacaatccgatgcagtggcagtattgttgaa
cctaaccaatccatgtcatgcagcatatcagattcatcaaatggctcaggcgcagtt
ctgcgtggaagctcatctacttccatggaagattggaaccaaatgagaacccacaac
agtaatagcagcgagagtggatcaacaacgctgatcgtaaaggccagttatagagaa
gacactgtacgtttcaagttcgagccatcagttgggtgtcctcagctctacaaagaa
gttggaaaacgttttaaactgcaggacgggtcgtttcagctgaagtacttggatgat
gaagaagaatgggtgatgctggttacagattctgatctccaagaatgtttggagata
ttacatggtatgggaaaacactcggtgaagtttctcgttcgtgatttgtctgcccct
ctaggtagttctggtggcagtaatggttatcttggaacaggcttatgacgtcgtaag
acatagacacacacagttatgtattcccagtgaaagaatgttgtttatttctctaga
tattagtatgcttataaataggcatgaaggagaaagacaatttggtatagtggagt
tcagcagaaaatgtatatgtttttcgttttatatgaatcagagaataaaagttgga
tgttatatctacgttgctaatgttgtacctgctcacccatctttcatataagaaaag
agaacactttagttatccctgtgatgcagaatcgtattctttgttatctctccatt
cctgtggaaaccaacaaagtcaactaaatttcggtttaattggttggttttaagtc
aacgaggacttgattttagttgggcttgggcctataattgtgttcatcattgggttt
tttccccccttatcagtttaacgtccatatccatatctttttctttttttaacggcaa
agttcatatccatatcttatgatgtgcctaaaagagggagaagatgcgaagacagaa
ttttcatatttgaaagggttcgatatcgatattgggaaacgaatcaaggtcaaaaaa
ctcagtctaatagttgaaatttaaaaatttattaattcaatccgattggtttcgtt
ttgttatggttcggttctatatcatcaaaccaatcggtttggtcctaaagataatta
taaatattcaccaacaccagtgttaaacacatatcaacaaacctaaagttagataaa
caaagaga**cccggg**

Arabidopsis Transmembrane Protein from Arabidopsis (clone#6468048)

cccgggaattggcactcttcttctgctgggttccaaaagaaacgaatcaatatgtgc
aacaagaagagctccagaagcagtcatcttctaaaatcttaatctaacaacagctca
agaagaaaaaaattccatagctagagagaacacaaagtcacaagacgacgtcgtaga
ggcacaaagtcaaacctgaatggcttaagccgaactgagtggttttgactagaccat
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tctctaaaggcccaatttcatttaggtttagtttatttgatcattatccttactata
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gtgaatgaaacaaaggcaacagtgaagttccagctgaagaaggttctgtgcatggga
gttgcagttggtaacctttt**cccggg**

Diaminopimelate Decarboxylase from Arabidopsis (clone#4159709)

cccgggtggcaaactgagatataagagggaaggtgattttcatgcaaattttttttt
tatttttttttgaatgaatgcaaaatttattcaaaaaaaaaaaacctgggctacatc
aagtacttcatttctgagttttgaaaaatctaaagacaacaaaagactttacaatt
taataaaaaaataataaaaatactttatcactctcaacgaaattgttgatttaataa
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aagtttttctgttttgttttatatctatagccaagaaagtttctcagatttacaaga
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gtgattaattttgagacaaattaaacatcgaattaaaagtaacatttggagggttta
tatgttatatatgtgacatgataagtccgattcatgactaatgtatatctggaatct
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tgtggtttttattttttgttagctagaaacttcagtgtttttttccgccaatggtag

tgctttgatgatggtccgg**cccggg**

Peroxidase from Arabidopsis (clone#4006885)

cccgggcaatcaaggtaacgaaggaggatcagcgaaaggatgggctatatttggagt
tttttcctgcgtgtaagtaatgctttgtgatcttccatgcggacatataactgaaga
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aaatacgttcttcttcccctcttatttcatatcactcaccacaaagtcttctcattt
cctcat**cccggg**

Mitochondrial Uncoupler from Arabidopsis (clone#4220510)

cccgggatgttgtgagtgaaggagaagaagagggaaacaaaggtatttatttgtagc
gagttttgttttgtgacgcggttttgtctgtgttcaatgttgacgaaacgagtgaga
gagtgtctgattattaaagaaaaccctaattaagtcagacccgccggttataaaaat
agtcaaaaagtaggaaaacgcgtgtgtgagtgagacagagacagcccattgtttgct
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cttccatcgattcctggcttctctctctcgctctctctgcatgtgctaaatcgccgg
actgatcctcactgtcacctctgtt**cccggg**

Stress protein from Arabidopsis (clone#6598614)

cccggggattaggggtttgagttgtcactggaaagaggtttgattgtgagtgatgat
ggagagattatgaaggagtttgtgtgtatttatagaggagttagggttttgaggttt
gatgagaagtaggtttgaagaagtttgttgttgcaacttatttagagttacttgtt
ccacaaccacaagtaagattggtcacttctaagttctaactagaaacaaccatgaca
catggagatttcagctaacctagtttaatgtatatgtattatatttatttaaatat
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gagaagaagaagaccaattactcaattagattttgttttgtggagcaattattgtct
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aacaagaagtaaagagttttcttatttccaattgtttttttaatctgatactttttttc
ataattttacaatgtttgatgaaaaaaaaacattcaaacctaaatttttcttttttttgg
tatgaattcaaacctgaattacttttgacgaggacccgacggtataaatagggtgat
ctcccaacaaacaaaaagggt**cccggg**

Pectinacetylesterase from Arabidopsis (clone#6671954)

cccgggtggtggggacaatggatccggtctgcgtagcaacaaggctgaaaaagatta

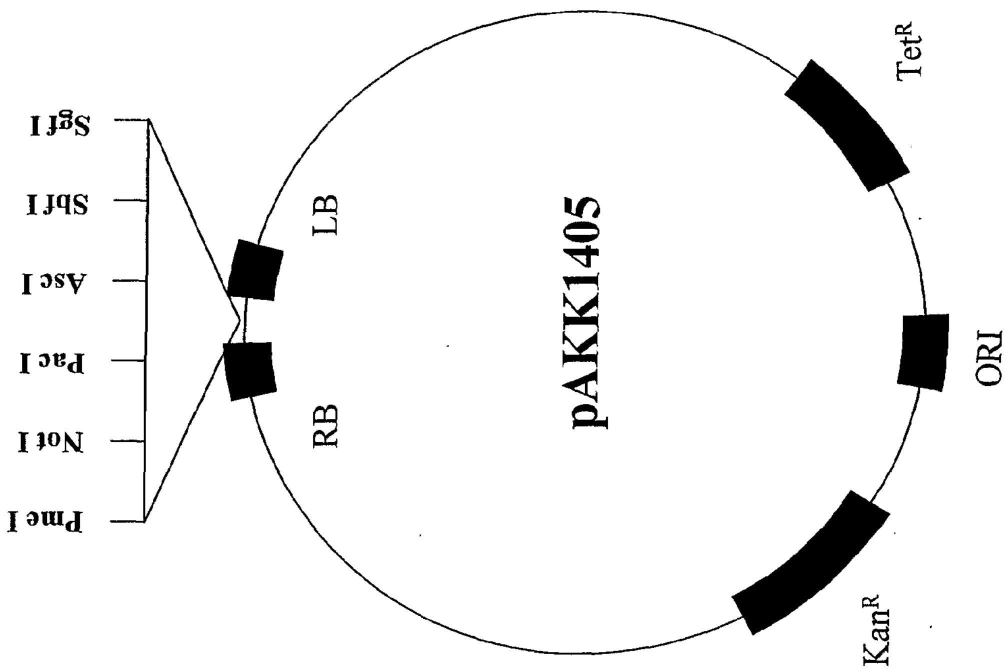
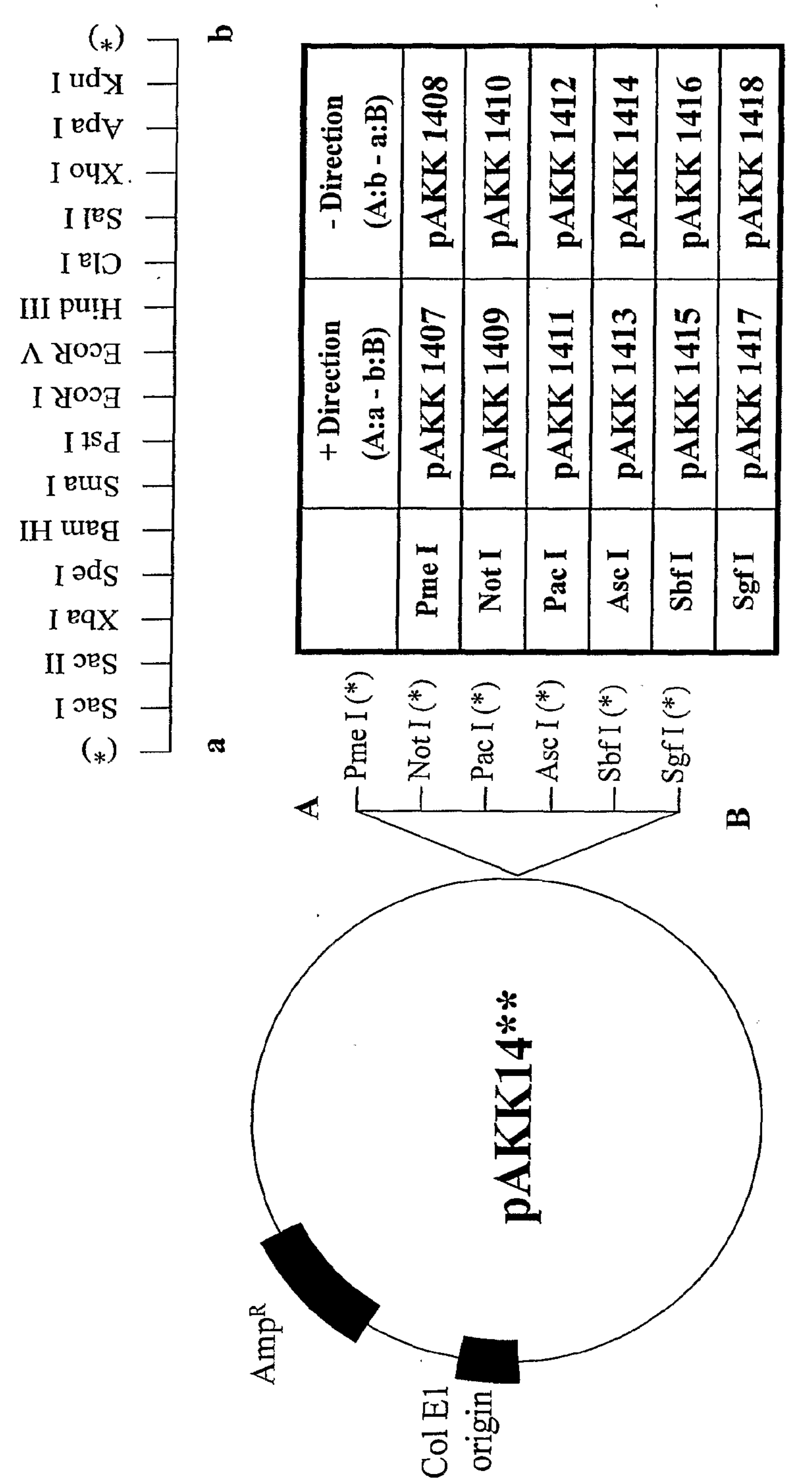


FIG. 1



	+ Direction (A:a - b:B)	- Direction (A:b - a:B)
Pme I	pAKK 1407	pAKK 1408
Not I	pAKK 1409	pAKK 1410
Pac I	pAKK 1411	pAKK 1412
Asc I	pAKK 1413	pAKK 1414
Sbf I	pAKK 1415	pAKK 1416
Sgf I	pAKK 1417	pAKK 1418

FIG. 2

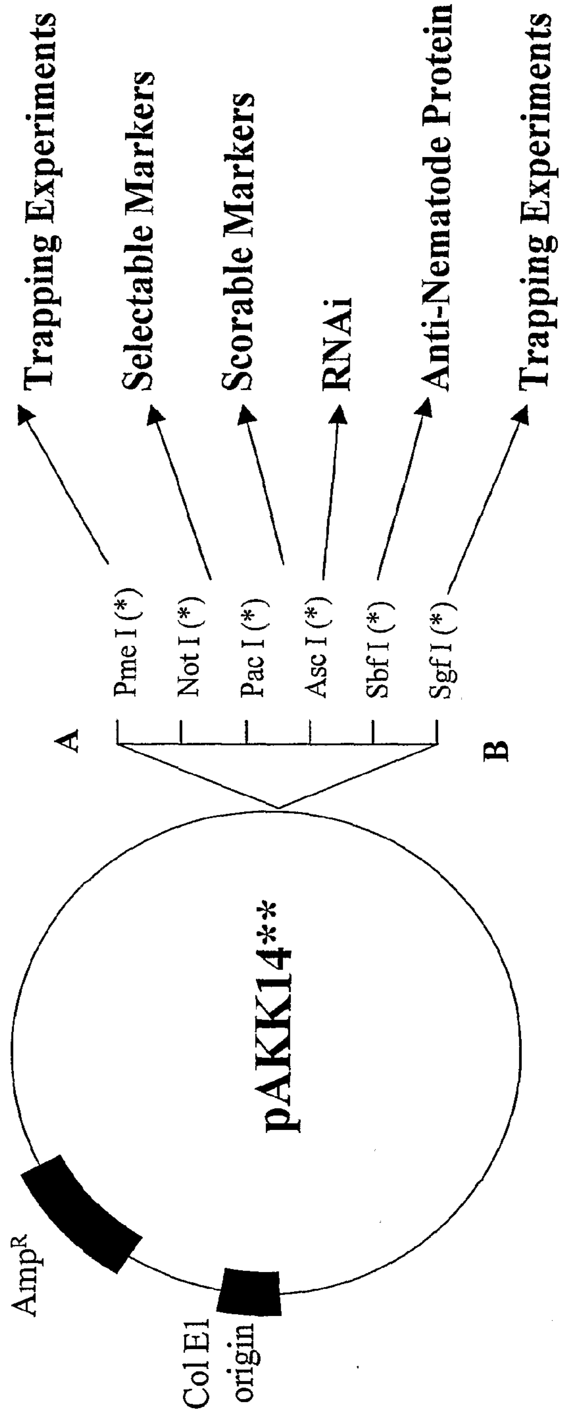

Trapping Experiments
Selectable Markers
Scorable Markers
RNAi
Anti-Nematode Protein
Trapping Experiments
Pme I (*)
Not I (*)
Pac I (*)
Asc I (*)
Sbf I (*)
Sgf I (*)
A
B
pAKK14**
AmpR
Col E1 origin

FIG. 3

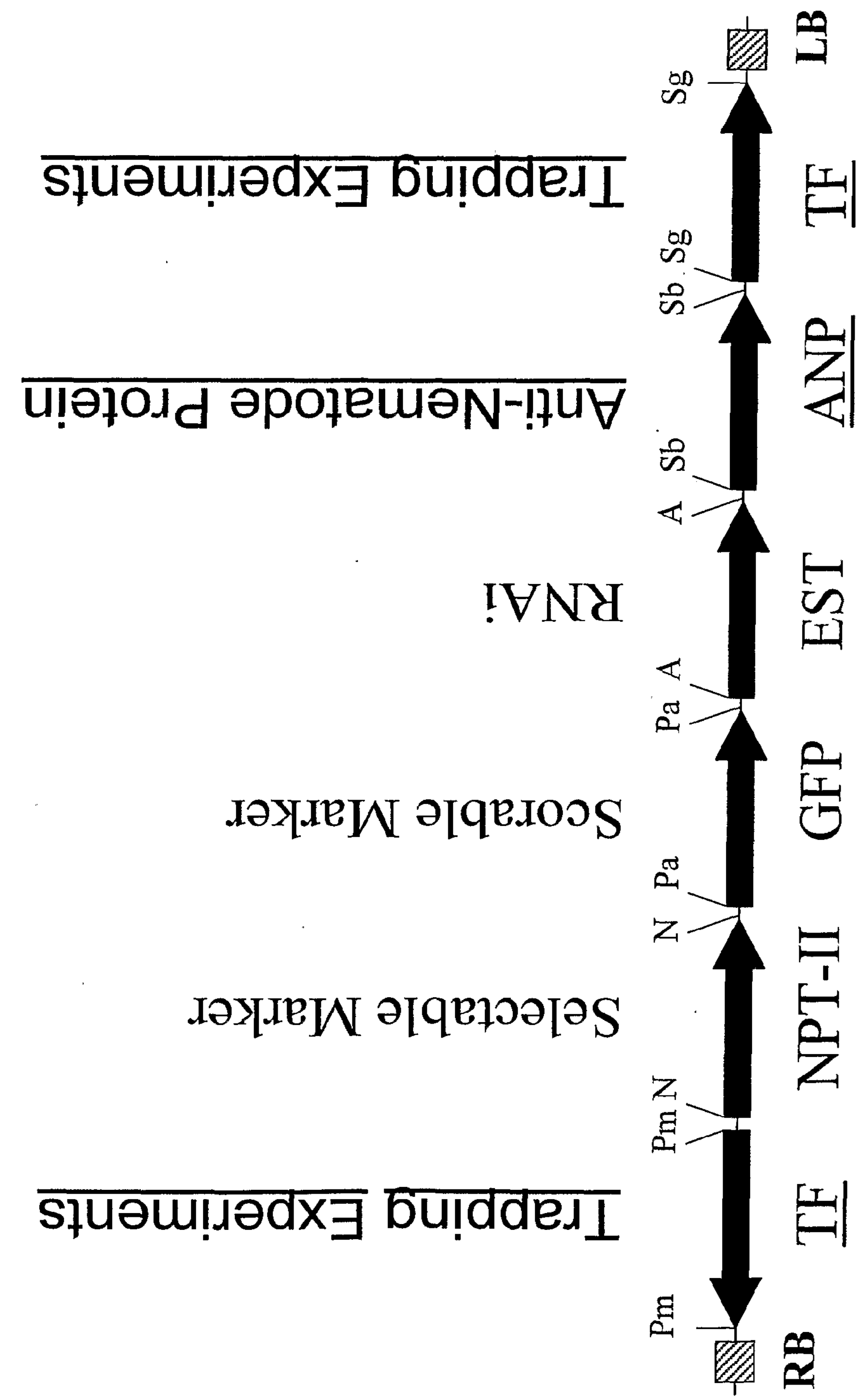

RB
Pm
TF
Trapping Experiments
Pm N
NPT-II
Selectable Marker
N Pa
GFP
Scorable Marker
Pa A
EST
RNAi
A Sb
ANP
Anti-Nematode Protein
Sb Sg
TF
Trapping Experiments
Sg
LB

FIG. 4

Selectable Markers

pNOS / NPT-II / tNOS

pSU / Bar / tNOS

pSU/ Intron / Bar / tNOS

pUBQ3 / Intron / PMI / tNOS

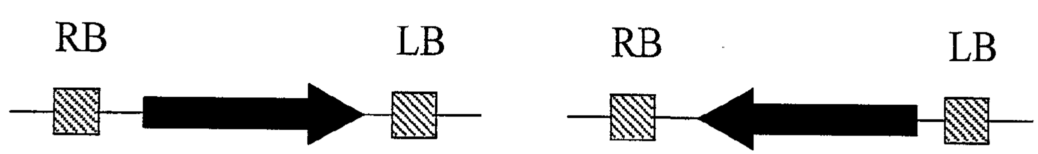


FIG. 5

Scorable Markers

Base Construct

/ Intron / Marker / tNOS [1]

pSU / Intron / Marker / tNOS [2]

pSBV1 / Intron / Marker / tNOS

pSBV2 / Intron / Marker / tNOS

Markers

GFP

GUS

NLS-GUS

PAP1C

[1] Construct useful for promoter analysis.
[2] Construct useful for high constitutive expression of genes of interest.

FIG. 6

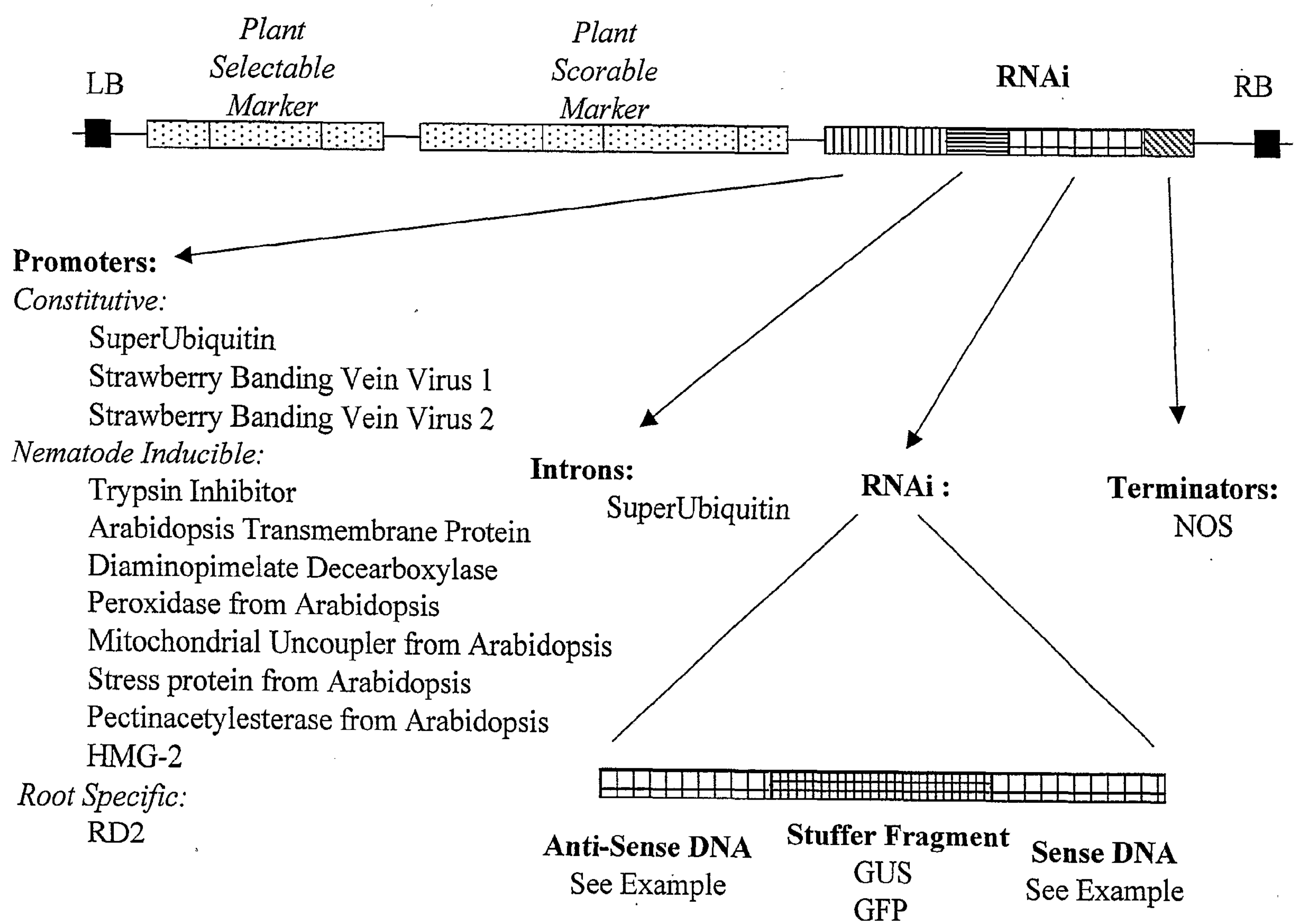
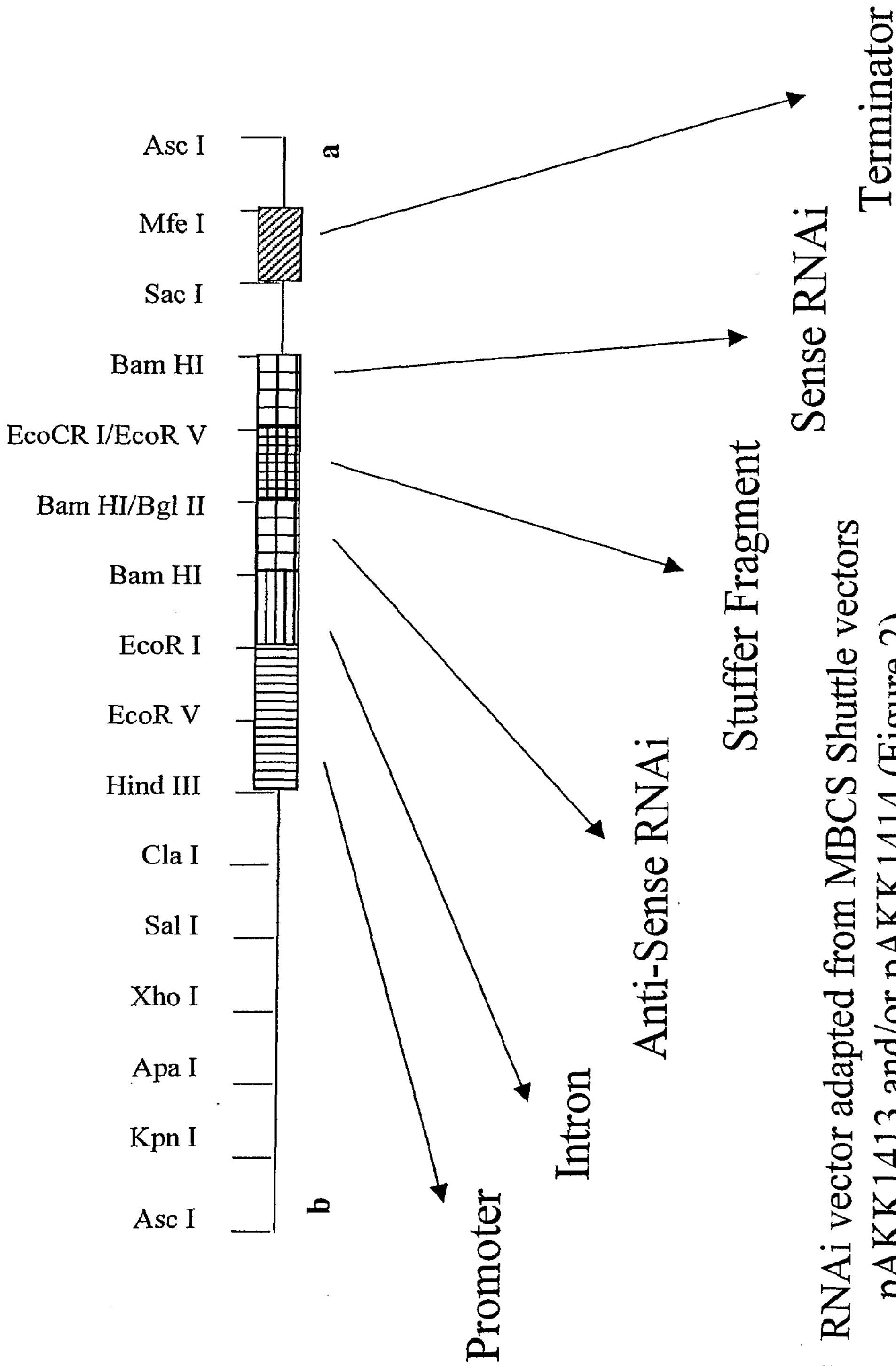

LB
Plant Selectable Marker
Plant Scorable Marker
RNAi
RB
Promoters:
Constitutive:
SuperUbiquitin
Strawberry Banding Vein Virus 1
Strawberry Banding Vein Virus 2
Nematode Inducible:
Trypsin Inhibitor
Arabidopsis Transmembrane Protein
Diaminopimelate Decearboxylase
Peroxidase from Arabidopsis
Mitochondrial Uncoupler from Arabidopsis
Stress protein from Arabidopsis
Pectinacetylesterase from Arabidopsis
HMG-2
Root Specific:
RD2
Introns:
SuperUbiquitin
RNAi :
Terminators:
NOS
Anti-Sense DNA
See Example
Stuffer Fragment
GUS
GFP
Sense DNA
See Example

FIG. 7



* RNAi vector adapted from MBCS Shuttle vectors pAKK1413 and/or pAKK1414 (Figure 2).

FIG. 8

AKK110P1
SEQUENCE LISTING

<110> Mushegian, Arcady R.
Taylor, Christopher G.
Feitelson, Gerald S.
Eroshkin, Alexey M.

<120> Materials and Methods for RNAi Control of Nematodes

<130> AKK-110P

<140>
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<160> 139

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<210> 6
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aaggaaaatg agaaga 136

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<213> Globodera rostochiensis

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aagtagccgt atttgcgaaa t 141

<210> 12
<211> 37
<212> DNA
<213> Globodera rostochiensis

<400> 12
gcgttgggtg caagctgtac acaaggtcgc ccggttt 37

<210> 13
<211> 161
<212> DNA
<213> Globodera rostochiensis

<400> 13
gcgcgttcca tcgcccgcac cacaaaaagt cccatcgctt catatcgtag cgcaaattgt 60
ctttggtgca aatggcaaaa cggccaaaat aatggtcgaa gccgtacaca accgccaccg 120
ccacagcgcc aaccccacac caaatgcgaa atttatcgaa a 161

<210> 14
<211> 306
<212> DNA
<213> Globodera rostochiensis

<400> 14
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agtttgatca ctcgattgtt agaagttcgg ggttgtagac cgggaaaaac agtgcaaatg 120
gacgaatctg agatacgcac tttgtgcatc aaaacacgtg aaattttgct gtcgcagcca 180
atcttgttgg agctcgaggc acctttaaaa atttgtggtg acattcacgg acaatataat 240
gatcttctga gattgttcga atatggtggg tttccaccgg aagcgaacta tctatttctt 300
ggggac 306

<210> 15
<211> 261
<212> DNA
<213> Globodera rostochiensis

<400> 15
gcaaagcctt gagacgattt gtttgctgct tgcttacaag attaaatatc ctgaaaattt 60
ttttcttctt cgtggcaatc acgaatgtgc ttcaatcaat cggatttacg gattttatga 120
tgaatgcaaa cggaggttcc tcaatcaagt tgtggaagac cttcactgac tgcttcaact 180
gtctgccaat tgccgcttta atcgacgaaa agatcttttg ctgccacgga ggctgtctcc 240
tgatttgcta aacatggcag c 261

<210> 16
<211> 151
<212> DNA
<213> Globodera rostochiensis

<400> 16
gaattctttg agtgcattca gcgtttaatt ttttcgtatt ataataagca tggctcgcgg 60
acccaaaaag catttgaagc gacttgcagc acccaaaaaa tggatgttgg acaaattggg 120
tggcgttttt gcgccacgtc cattgtgcgg a 151

<210> 17
<211> 306

<212> DNA
<213> Globodera rostochiensis

<400> 17
tcaagtacgc gcagtcgtac aacgaagcgc gcatgatctg caaacagcgg ctgatcaagg 60
tggacggcaa agtgcgcacc gagatgcgct tcccgtgcgg aataatggat gtgatctcga 120
ttgagaagac aaacgaaacg tttcgtctgg tgtacgatgt gaagggccgt tttgtcatcc 180
atcgaattca aaagctggag ggccagtaca agctgtgcaa agtgaagaag caggccgtcg 240
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ctcatc 306

<210> 18
<211> 528
<212> DNA
<213> Globodera rostochiensis

<400> 18
gaattcgcac aacgaattga agacttatgc ggcagaaaaa ggacttttgc caaagtgtga 60
ggagcaagca gacgaccttt cggattggct ttgttcgtcc attgggttgg agcatcgccc 120
gttcctaccg tatacaaacg ctgtaataaa tgaaacaatt cgattagtca atttgatccc 180
gttcaatctt agccatttgg cgcttgaaga tatgcaaatt ggcaatttta ttgtgaagcg 240
tgggacacca attgtaccgc aggtcagcag tgttctgttc gacgaaaaac tgtatccgga 300
gcccgatcgg tttttgcccg aacgctttct ggacgatgag ggccgtttga agaaaagcga 360
cgaacttatt gcatttgggg ttgggaaaag gcaatgtgcc ggcgaagctt tggcccgaat 420
gacacttttt ctgtttgccg ctaatttctt tctcgcctac aaagttctcc cgtccgatcc 480
actgaatcct ccaagcctga aaaagttggc ggattatctg tttacaca 528

<210> 19
<211> 335
<212> DNA
<213> Globodera rostochiensis

<400> 19
gaattctttg agaaagcgga aattcgtttt tggctataaa atgattctgt gggccacgat 60
tttgttgatg gctttggaca ttgcgttcgg tggcaccaat caaatggaat ttgatcagtc 120
ggcgccgatg ttccccgact cccagttcat cgatttgatt tcgcgcgaca tcgaatcctt 180
ctccggccca ttgggcgttg gccataaatt tatgagcggc ggtgccggtg agggcgtcca 240
acagctaggc cccgaggggc cctttgagca gcggcaacag gtgaagagtg acaatgttct 300
ccccgcgtat tgcgagcctc caaatccctg tccga 335

<210> 20
<211> 52
<212> DNA
<213> Globodera rostochiensis

<400> 20
ggacggctgc acggaacagt tcgagaacac tgccgagttt tcgcgcagct ac 52

<210> 21
<211> 190
<212> DNA
<213> Globodera rostochiensis

<400> 21
gcttgtgtga ccaggagcac atgtttaact gtccgtcgaa gaacaaccgc gaggagtacg 60
agcaggatct ggagcaattg ctggccaaca acggactgca caaatcaatg attgccaaga 120
aattccatct cacgcgggcg gaggagccgc gccgtcgaaa acgctcttgt cgcccggctt 180
cggccaaccg 190

<210> 22
<211> 52
<212> DNA
<213> Globodera rostochiensis

<400> 22
ccgctacaac ccctacctgg agggcgcccc gctgaagtca gtggccaaaa ag 52

<210> 23
<211> 54
<212> DNA
<213> Globodera rostochiensis

<400> 23
gaattccgac tctcaaggtg gacccacgcc caaccaacag caattgtcag ctgc 54

<210> 24
<211> 77
<212> DNA
<213> Globodera rostochiensis

<400> 24
ccgcacatgt cgaggcctcc atcttttggc actggtcatc accttccgcc tactgctaac 60
aacagaccgg aacagca 77

<210> 25
<211> 439
<212> DNA
<213> Globodera rostochiensis

<400> 25
gtcaatcaaa aacgccgact tcgattcctc agctgatggt cagtaatgcg ctaaagggca 60
tccattccgt ctcttctaca tcagcaacac aatcacattc cacgcccagt tttatgacac 120
acaacgtgca gcagcaacat gttgttggtc aacaacagca gcaacaacag aatttccaac 180
aaccgccgcc cctatcgtac actcacagcc accaacaaca aaaacaacca ccacaagcgt 240
cacagtcgat gttgtcaatg aaaagtggca atgttgtcgt tgttgttccg caacaatcgc 300
agcagcacca ctaccaacag cggacactga cgccactgaa gcacacatcc gcatcctcca 360
cgtccgatcg cttcgtcatc accaaaacca acagggtgct tccactcccg tcgcagcaag 420
gcgccacggc cactgatga 439

<210> 26
<211> 539
<212> DNA
<213> Globodera rostochiensis

<400> 26
gaattcgttt gagacacatc caattaatta atagttattg ttggcaatgg gacgagttcg 60
aacgaaaact gtgaagaagg cgtcgcgcgt cattattgag aagtattaca ccaaattggg 120
cctcgacttt cacaccaaca agcgcatttg cgaggaggtg gccattatcc caagcaaacg 180
gatgcggaac cgaattgcgg gatttatcac acatctgatg aagcgcattg agctgggccc 240
tgtccgtggc atttccatca aattgcagga ggaggagcgc gagcgtcgcg acaattacat 300
gcccgaaatc tcttacctgg atgcgcagaa tcaccagatg atcagcaccg accaagagac 360
gaaggatatg gcggaatttc tggggctagg cctcaacttg gaagtgaaag ggcctttgac 420
gagtggcggc gctggcgcag gacgtcgttg agtcaggaca attggcatta ttgttgaaaa 480
atcatcgatg ttttgttcgc atttggatga taatgcgctg ataaattttt gttgatttt 539

<210> 27
<211> 179
<212> DNA
<213> Globodera rostochiensis

<400> 27
gaattcnaca gtttctgtga gtaatggcat ntcacactgc cggcatccaa cagttgcttg 60
cggccgaaaa gcgtgcggca gaaaagatta atgatgcccg gaagcgaaaa gcacagcgac 120
ttaagcaggc caaacaagaa gcccaggcgg agatcgagca gtatcgncag gagagggag 179

<210> 28
<211> 133
<212> DNA
<213> Globodera rostochiensis

<400> 28
gcaaaattat ttgggcacgc gcgacgacat cgagcagcaa ataaagcgcg agacagaaga 60
gtcgctggag gcaatgaatc gcaatgtcgc ggcgaacaaa cagcaggtca ttgtacgtct 120
gctgcagttg gtg 133

<210> 29
<211> 482
<212> DNA
<213> Globodera rostochiensis

<400> 29
gaattcgtga aatcaaaagc ttttttaatt tatttacaca aaaaatggtt ccaccaccaa 60
ttcgcgtgtt ggtcactggt gccgctggac aaattggcta ttcactggtt ctgcaaatcg 120
caaaaggcga tgtgtttggc aaagatcagc caattgttct cgttctcctc gacattccac 180
cgatggccga agtactctct ggtgtccatt ttgaattgat ggactgtgcg ttggcaaacc 240
ttgccggtgt ggaggctgtg accacggaag agcaggcctt caaggacatt gactacgctt 300
ttcttgtcgg agcgatgccc cgaagagagg gaatggaacg aaaggacctt ttggcggcaa 360
atgtcaaaat tttcaagtcc caaggcgaag cattggcccg cttttccaag cccgtncgtc 420
aaagttctcg tggtgggcaa cccggccaac acgaacgcgt acatttgcgc aaaatatgcc 480
gg 482

<210> 30
<211> 605
<212> DNA
<213> Globodera rostochiensis

<400> 30
gaattcaaag tgccgaaagc gttcaaaata attccggcaa tggcaaattg ggaacaaatt 60
ctagacctta cttcccccga aaaatggagt tcagcggcga tgtttcaagc aactcgtgtg 120
ttttctgcca ccggcacacc gtcacaatgc caaaggttca acactttggt gctgttgcca 180
cgactccgtg atgagattga cgagtacaag aagctaaact ttcatttgta tcagtgcttg 240
tttaaagcaa tgttcaagcc ggccggattt tttaagggca ttattttgcc tctttgcaaa 300
tctggcactt gcactctccg tgaagccatc atctttgggt ctgctctgcg aaagatttca 360
ataccgcaac tccacgccgc tgcagcaatg ctcagcatag caaaaatgga ctactcgggc 420
gccatttctt ttatcctacg tgttcttgtt gaaaaaaatt acacacttcc tttccgagca 480
ttagacggcc tcgtttttca ttttcttgga atgcgctcac atcagggcga gctgccagtg 540
atttggcacc agacactgtt ggcttttgtc gagcgttacg caaaagacat aagtgcagaa 600
cagag 605

<210> 31
<211> 112
<212> DNA
<213> Globodera rostochiensis

<400> 31
ccattcccat catcaaatta ccccgattta ctgcggcttt tgcgcggcgc cgagtcgagg 60
aatgaggaaa gtgaagcaaa tgtgcccgtt tatgcgcgta atgatgaaat gg 112

<210> 32
<211> 105
<212> DNA
<213> Globodera rostochiensis

<400> 32
gaattcgttt gagcatttat ttgacaaaat ctgaataaat ggccgtacca aaagaagtta 60
ttgacaaaat cgaggcgggt tacaagaagc ttcaggaagc gtctn 105

<210> 33

<211> 425
<212> DNA
<213> Globodera rostochiensis

<400> 33
aagaagtacc tcaccaagga agtcgtcgat gcctgcaagg ataagcgcac caagcttgga 60
gcgaccttgc tggatgtgat ccagtcgggc gttgccaact tggacagcgg agttggggtg 120
tacgctcctg acgctgaggc ttacaccttg ttcaagccgt tgttcgaccc gatcatcaac 180
gactaccatg gtggctttgg tccgggcagc aagcagccgg caactgacct tggtgacggc 240
aaaacgcana tgctgaccgg atctcgaccc cgaggggaaa atttatcaat ttcgacacgc 300
gttcgttgcg gccgtttcct ttaagggata cccggttcaa cccgtgcttg acnaaaggan 360
aactacnttt ggagatggga aacnaaggtc nagggccgtt ttctaacatt ttnaagggcn 420
atcct 425

<210> 34
<211> 581
<212> DNA
<213> Globodera rostochiensis

<400> 34
gaattcgttt gagcgaagag ttttgtggtt gacaccggtt tatggacttt tagcccgtga 60
tccttgacgg tccaaagccg cgttcagttc cgtgccgtgt tttttaaaag aggcggagag 120
tttgacggtc attccaagca gccaataaac caccaaaacc aaataccccc ccccaatcga 180
tcccccccct ccaattcctc cgcattattc gcattatcaa ttctaatcag cacaaccact 240
gcatcattcc tttcccgacc atacgatgct aagtgaaact ttgaaaattg gcttcatcgg 300
agccggaaag atggcccaag cattggcaag aggacttatc aattcggggc gatacccggc 360
agagaatttg atggcgagtt gtccaaagac ggacgaggct ttactggagc aatgcaaaaa 420
attgggaatc ggaacgacgc acgacaacac tttggtcgcg cgagagaacg acgtcatcgt 480
attggcggtc aagccgatgc acatcagcaa agtgacgtcg gaaatcgcac ccaatttccg 540
gagggaacat ttgcttattt cattgattag gaattacact t 581

<210> 35
<211> 102
<212> DNA
<213> Globodera rostochiensis

<400> 35
gaattcgttt gagaatttta ctttatataa ttgacgttta atcagcagcc ataagcaatg 60
cccatcaaag catccggaga aacattaagg aagtttattg tc 102

<210> 36
<211> 34
<212> DNA
<213> Globodera rostochiensis

<400> 36
tgcaaatgat gcaaacccca cgcttcacaa gatg 34

<210> 37
<211> 100
<212> DNA
<213> Globodera rostochiensis

<400> 37
tcatgttgtg gccaaatctc gcttctggta ctttacgagc atgctgcgtc gagttaagaa 60
aacacacgga gagatcgttt cgtgtcaaga ggttttcgag 100

<210> 38
<211> 176
<212> DNA
<213> Globodera rostochiensis

<400> 38

tgaagaactt cggaatttgg ctccgttacg attctcgtac tggacaccac aatatgtacc 60
gcgagtatcg ctgatgttac cgaggccggt gccgtgaccc aatgctatcg cgacatgggc 120
gctcgtcacc gcgctcaggc ggatcgaatt caaatcatca aagtgcaaac ctcaag 176

<210> 39
<211> 155
<212> DNA
<213> Globodera rostochiensis

<400> 39
gaattccaag tttgaggtat tgtttgttat acgatttctt acaaatgaca gaacaaactg 60
agcgcgcgtt ccaaaaacaa ccgatcgttt ttctgaacga caagttcaga acgcaaggga 120
ttgggaagaa ggcatccaac aaggaccgtt actgg 155

<210> 40
<211> 35
<212> DNA
<213> Globodera rostochiensis

<400> 40
tcctcgcgag gctattgagg gcatatatat cgaca 35

<210> 41
<211> 70
<212> DNA
<213> Globodera rostochiensis

<400> 41
tggaaatgtg tccatccgcg gtcgcattct cactggggtg gtgatcaaaa acaaaatgca 60
gcggacgatt 70

<210> 42
<211> 85
<212> DNA
<213> Globodera rostochiensis

<400> 42
tcgtaccaaa atatcgtcgc tatgagaaac gccacaaaaa catgtccgtc cactgttcgc 60
cgtgcttccg agatgtctct ctcgg 85

<210> 43
<211> 193
<212> DNA
<213> Globodera rostochiensis

<400> 43
agttcggttc aatgtgctca aggtgatcaa agcatcgggc tcgaagaaag cgttcgacaa 60
attctgagtc ggccaagcca accgcgaacg gtcatttgtt atggttccta attgttgctg 120
tttttcaatt atttgtgtta aatgactgaa tttatgatca acggtatact agtattcttc 180
tgaaaaagct cga 193

<210> 44
<211> 219
<212> DNA
<213> Globodera rostochiensis

<400> 44
gaattcattt agatttgttt tgaagctaga aatctttatt ttgggagtca acgacaatgg 60
gaagacgtcc ggcgcgttgt tatcgctata ttaagaacaa gccgtatccg aagtcgcgct 120
tttgtcgcgg tgtacccgac ccaaaaattc gcatttttga tttgggtaga aagcgcgcca 180
ccgttgacga attcccatgc tgcgtgcata tgatatcga 219

<210> 45
<211> 489
<212> DNA
<213> Globodera rostochiensis

<400> 45
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aaggacgggt ttcatatgcg cgtcagaatc catccatacc atgtaattcg catcaacaaa 120
atgttgtcct gcgctggtgc ggaccgtctg cagactggga tgcgtggtgc gttcggaaag 180
cctcagggac tcgtggcgcg tgtcagcatc ggtgatatgc tgatgtcagt gcgtattcgt 240
gaccaacacc aagctcacgc attggaggcg ttccgtcggg ctaaattcaa gttccctggt 300
cgtcaataca tcgtcttgtc ccgcaagtgg ggcttcacca aattcgatcg cgaggtatac 360
gagaaatacc gcaaggaggg ccgtgttatc cctgacggtg tgcattgcaa gttactcaag 420
caacacggac ccgctgaagg agtggctcaa gaaccccatt taatcttctg tttgtcttgt 480
gactcttgg 489

<210> 46
<211> 101
<212> DNA
<213> Globodera rostochiensis

<400> 46
gaattccccg gctcgagccg ggttgacgat gtcctcctcc acctcctctc actgcgttcc 60
gtcctccttc agccggaaat tgttcctgtg gctgttgccg g 101

<210> 47
<211> 485
<212> DNA
<213> Globodera rostochiensis

<400> 47
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tcgttccgat gacgtcgttt ggccaaccgt tgccccccgtc accgctttca ctggtgccaa 120
acccgccgct ttattttgtg ttcccagaaa acttgccgtt ggagcggccc ttcgacgagc 180
aaaacgacgg ctccgaggag gaattagccg aagaagcgat gggaacgaag gcgaagaggg 240
cgcaaacgtt cgtccgattc ggcaaaaggg cgcaaacatt tgtgcggttc ggaaagcgtg 300
cacaaacatt tgtacgcctc ggaagggaca cgcaaaggca attcgatggg aaaatgcaaa 360
gtgaacagca acagaaaaag gcttaaagca aacggcggcg acttttcttt taatgaatgc 420
gcgcccaccg catgacaatt cttttgtgta atgtgttgcg atttttatga tcggtaaatg 480
taaca 485

<210> 48
<211> 651
<212> DNA
<213> Globodera rostochiensis

<400> 48
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ctgctggaaa gacgaccatt ctgtacaagt taaagctcgg cgaaattgtc accaccatcc 120
caacaattgg cttcaacgtg gaaaccgtcg aatacagaaa catctcgttc actgtttggg 180
acgtgggtgg tcaagacaaa attcgtccac tttggaggca ctacttccag aacacgcaag 240
gactgatctt cgtcgtggac agcaacgatc gcgagcgtgt gggcgaggcg cgtgaagagt 300
tgatgcgaat gctggcggag gacgagttgc gcgacgcggt gttgctggtg ttcgctaaca 360
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acttgcgaaa ccgcaattgg tacatccagg ccacctgcgc gacttcgggc gacggactct 480
acgagggact ggactggctg agcaaccagc tcaagaacag aggctaagct gggttggtgt 540
ctgttgcact tgcccgcgga attgatgacg attgaattta tttgtgtgtt tgcgcgcgca 600
gctcttttgt gggacgtccg attaattttg ataattattt tattccgtgt t 651

<210> 49
<211> 660
<212> DNA
<213> Globodera rostochiensis

<400> 49
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cccaactgag atcaaaatcg tgtacctgcg ttgcgtcggt ggtgaaattg gtgcaacatc 120
tgcacttgca ccaaaagttg gcccacttgg attgtcgccc aaaaaaattg gtgaagacat 180
tgcgaaggcc acacaggact ggaaagggct taaggttacc tgcaagctga caattcagaa 240
tcgtgtcgcc aagatcgacg ttgtcccatc ggccgcctct ctgatcatca aagagttgcg 300
cgaacctccg cgagaccgca aaaaagtcaa aaacgtgaag cacaatggca acctgaccat 360
cgagcaagtg atcaacattg cgcgtcagat gcgccctcgt tcaatcgcac ggaagttgca 420
gggcaccgtg aaggaaattt tgggaaccgc ccagtcggtt ggctgcacca tcgatggaca 480
acatccgcac gacattgtgg acgcgatcag agggggagac atcgaaatac ccgaggaata 540
aagaaaggac ggcgcctccg atttttgtgg gacggacatt gggaatttga ggtgaatgag 600
ttgccaattt cattcattca tcaattgttg ttattgntgg tacggataaa tttgtaattg 660

<210> 50
<211> 625
<212> DNA
<213> Globodera rostochiensis

<400> 50
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gtggcaatcc gggacggcgt cccctacccc ccactgcctc ctacaaaccg atcccccgaa 120
tacatgaaca tgctgacccg ctccttctcc gtgccaaatt tccgcatcta ctcgggcgcc 180
atcggaccgt acagaccttc gttgcccgtg tacacttaca acacttacca cgggtacttc 240
ccctaccgca actaccgcgg ctacaccttg gcgaatgctt actggtacga ccgatactat 300
tacttctcgc cgctgtacaa acgaagcatg ttccccaccc gcttcaaaca ttgtgactat 360
aaagcgaacc cgcactattg gcactacccg cacacctttt gggactatcc ctaccagggc 420
aaatggttcg actacgacaa ccctcccaat taccggccct actacaacca tcgccttaac 480
ggatatgctc ggccgtatca ctaccggtcc catgcgctgg cccacccgtt caattacccg 540
gaaggaatgg tcaggaaacg ggtctgacaa atcgaactgc tccaaattga cgtggtccgc 600
attcgaaaga agacgaaaaa agctt 625

<210> 51
<211> 402
<212> DNA
<213> Globodera rostochiensis

<400> 51
gaattccaag tttgagcaac attttgaaaa tgaccgaagc caaaaaactt cccgaggtgc 60
cggaaacttt gctcaagcga cgcaaaatca gagctgcgca aaaggccgca aaagcaaaga 120
acaaattgag ttctatcaaa aaagcacgga ccaagaaggt ggaaatcttc aaaagagccg 180
agcagtattt ggtggagtac cgtcagaagc aacgccaatt gcttgcgctg aaacgtgaat 240
cgaagaaagt cggcaattat tatgtgccag aagagcccaa actcgccttt gtggtccgaa 300
tcaaaggcat caataagatt catccgcgtc ctcgcaaggt tctgcagctt ctccgcttgc 360
gtcagatcaa caacggcgtt ttcgtaaagt tgaacaaggc ga 402

<210> 52
<211> 433
<212> DNA
<213> Globodera rostochiensis

<400> 52
ccgacccgta catcgcttgg ggttatccga gtcagaagat catccgtcag ttggtctaca 60
aacgcggtta cgccaaagag aagggacagc gcattccaat aacggataac aacattgttg 120
agcgcagttt gggcaagcat gacgtgattt gtgtggagga tatgatccat cagatttgga 180
ccggtcggac cgcacttcaa acaggtgacc aacttcctat ggcctttcaa gctgagcaac 240
ccggtgggcg ggttcaagaa gaagtccaat cacttttgtg gagggaggcg attatggaaa 300
ccgcgaggac caaatcaaca aattattgga aagaatggtc taatggaagg gaagcggana 360
aagaaaggaa attgnggcgt ttttctgttg ttgttttgac gataaattgt taactccaaa 420
aaaaaaaaaa aaa 433

<210> 53
<211> 768
<212> DNA

<213> Globodera rostochiensis

<400> 53
gaattcgttt gaggtcaaac tttattagcg tatttaacaa tgtccgaagg aggagcgaaa 60
aagagtagca gcggtgccaa gggggggttt gatgtcaaga aatttgcgat cgatcttgcg 120
tccggtggta ctgcggcggc tgtctccaaa actgttgttg ctcccattga acgtgtcaaa 180
ctcttgttgc aggtgcaaga tgcttccgct cacatcactg ccgacaaacg ctacaaaggc 240
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aacttggcca acgttatccg ttatttcccg actcaagcgc tgaacttcgc cttcaaagac 360
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gtcatgaatt tggcctctgg aggtgcggcc ggcgccacgt cgctgacctt tgtttatccg 480
ctgggacttt gcccgtacgc gtttggcccg tcgatgtccg aaaagctggt tcccgcgagt 540
tcaacggttt ggcccactgc atcgcaaaaa tcttcaagtc ggacggtccc atcggtcttt 600
accgcggctt cttcgtctcc gtccagggca tcatcattta ccgcgccgcc tactttggat 660
gctttgacac cgcgaagatg attttcgcgc cggatggcaa gcagatgaat ttcttcctca 720
catgggccat cgctcaggtc gtcaccgtgt cgtccggtgt cctctcct 768

<210> 54
<211> 338
<212> DNA
<213> Globodera rostochiensis

<400> 54
gaattccagc agattaattg gaatggctga gaacatcgaa gagattcttg ccgaaatcga 60
cggctcccaa attgaggagt atcaacgctt tttcgacatg ttcgaccgcg gaaagaatgg 120
ttacattatg gccacccaaa ttggacaaat tatgaacgcg atggagcagg actttgacga 180
aaagaccctc cgaaaattga tccgcaagtt cgacgcggac ggttccggca aactggagtt 240
cgacgagttc tgcgcgttgg tgtacacggt ggccaacact gtggacaagg acactctgcg 300
aaaggagctg aaggaggcat tccgactctt tgacaagg 338

<210> 55
<211> 267
<212> DNA
<213> Globodera rostochiensis

<400> 55
gaaattgcgc ccgatctcag cgacaaggat ttggaggcgg cggtcgacga aattgacgag 60
gacggcagcg ggaagatcga attcgaggag ttctgggagt tgatggcggg cgaaaccgac 120
tgagaaaaga gcaaatcgat ccaaatccaa acggacccgt cccatttcac ctccatccgt 180
ccgtcgtatt attatatttt ccagtggaat tttcccatta aaattcggtg aaagtaaaat 240
aatttgacga aaaaaaaaaa aaaaaaa 267

<210> 56
<211> 597
<212> DNA
<213> Globodera rostochiensis

<400> 56
gaattcgctg gacacttcgc atccggagta cagccacgag cagagcatcg accagaccag 60
catcccctac cagatgggtt cgaacaagta cgcctcgcag aagggcatga ccggctttgg 120
acagccccgt tgggaggtgc ttgacccgtc catctcgtac cagaaccgca agtcgcaagg 180
aatggttcgt ctacagtcgg gtaccaaccg gttcgcctcc caggcgggca tgaccggctt 240
cggcacaccc aggaacacca cctatgaggc ggaggcaggc gagctgccct acgaggacat 300
gaagaagtcg gaggcgatca tcccgtccca ggccggttgg aacaagggcg actcgcagaa 360
gttgatgacc aacttcggca cgccccgtaa caccaccacc aaggtcaaag tggagaattt 420
ggcggaaatt ccggaggaca ttttgctgaa aggacacggc gaggtgcgcc tgcagtccgg 480
taccaaccgg ttcgcgtccc agaagggctt cgtcgcgttc ggtaccggac gtgacgtgtg 540
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<210> 57
<211> 80
<212> DNA
<213> Globodera rostochiensis

<400> 57
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ttcggtacgg gcccgtcgtg 80

<210> 58
<211> 513
<212> DNA
<213> Globodera rostochiensis

<400> 58
gaattcgcca caccgctcac atcgcgtgca aattcgccga acttaaagag aaggtggacc 60
gncggtctgg caagaaagtt gaggacaacc cgaagtcgct gaagactggc gacgccggaa 120
ttgtcgaact gattccgacc aagccgatgt gtgtggaggc attcactgac tacgcaccgc 180
tcggccgttt tgctgttcgc gacatgaggc anactgttgc cgtgggcgcg atcaaatcag 240
tggagaagac ggaaggcggt ggcaaagtga ccaagccagc gcagaaggtc ggcgcgactg 300
gtggcgggaa gaagacatga ccaaggggag gggcggttcc ctaagggcca accgtcgacg 360
aaaatgcgac caacctcttg tttatcgttg tcttattcag ttccttccac ccgtctctat 420
ccatattgtc gttgcgttgg ataatgtttt attttttgtt attgtcctgg ttggaaaata 480
aatttggtca attaaaaaaa aactcgtgcc gaa 513

<210> 59
<211> 393
<212> DNA
<213> Globodera rostochiensis

<400> 59
gaattcgttt gagcgaaaaa aacatactat acaatggcaa caactgagaa gcctcaggtg 60
gttcaacagc ccgtgcaggt ctttggccga aagaagacag caacagccgt tgcgtttgca 120
aaaaggggca agggcttgat caaggtcaat gggcgtcctt tggactacat gcagccggag 180
attctgcgca ttaagctcca ggagccaatt ctcattgttg ggaaggacaa atttgaggga 240
atcgacatac gaatccgcgt caagggcggt ggacacattg cgcaaattta tgcaattcgc 300
caagcactgg ccaaggcact ggtcgctttc taccagaaga atgtcgacga gcagagcaaa 360
aaggaactga aggagcaatt tgttgcttac gac 393

<210> 60
<211> 154
<212> DNA
<213> Globodera rostochiensis

<400> 60
cacgagccaa agaaattcgg tggacccggg agctcgcgct cgctaccaga atcgtaccgt 60
taagaaataa ttttgtagat caaatgtttt gatgatgatc cttgtttttg ttgttgataa 120
aaaaaattta taaaaaaaaa ccgccgatac tgac 154

<210> 61
<211> 666
<212> DNA
<213> Globodera rostochiensis

<400> 61
gtattccaag tttgagcgat cagagttctt caatctatta tcaactgttt tccatcaacc 60
aactgtcatc atgcaaattt tcgtcaagac gctcaccggc aagaccatca ctctcgaggt 120
cgaggctagc gataccatcg agaacgtgaa agccaagatc caggacaagg agggcattcc 180
gcctgatcag cagcgtctga tcttcgccgg aaaacagctt gaagacggac gcaccttggc 240
cgactacaac atccagaagg agtccactct ccatctcgtg ctgcgtctcc gtggcggaat 300
gcaaattttc gtcaagacgc tcaccggcaa gaccatcact ttggaggtcg aggccagcga 360
caccatcgag aacgtgaagg ccaagatcca ggacaaggag ggcattccgc ctgatcagca 420
gcgtctgatc ttcgccggaa aacagctcga agacgggcgc actctggccg actacaacat 480
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gggctgatgg aaagatgacg aatatgatgt ctattcgatg acttgtctct ttcgatataa 600
ttgattgtgt tccatttgtc ggtcatcaaa tctttatgac cccctcattg ggcatggaac 660
gataaa 666

<210> 62
<211> 213
<212> DNA
<213> Globodera rostochiensis

<400> 62
gaattcgttt gagaaacttt ttcaaccatt cattcaaatg tctcatcaag tgacacgggc 60
agcactcaac cacgggacgc gtgtactgag cgtgttggag aaggtcaagt tggtctgctg 120
gtttgaggag acacattcgt tcgcgcaagt ggctcgaaga taccgggcag aatttggtat 180
ggaaccaccg cagttggacc aagtgaagaa gtt 213

<210> 63
<211> 488
<212> DNA
<213> Globodera rostochiensis

<400> 63
agcaccggct caatcctcaa tggcacaacg acggcattct ccggcatagg agacggagtc 60
ggtcttggag aacaacagcc aattcccgtc gtaagcgatg cgggactgga tgcggaagaa 120
cagctgagaa tggccagaat gtgagccgga ggacctgaag atttatgaac gaaattttcc 180
agtgaagtgg accaacgctc ttcgacttta tctgctttgt gtaaagtgta tagaatcggc 240
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taagcgtgaa taatttattg atttgttttt tctttctttt atctccgcct cgaagtcgca 360
agtgttcctt ttggcccgtt cccttttgtt ttgaatgtta ttccattccc atcccctcac 420
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tgcgattg 488

<210> 64
<211> 249
<212> DNA
<213> Globodera rostochiensis

<400> 64
wccrgakbng aacahcdkdg vhwatnvcbn gschvbwagc rngtcsvddb wgnhnsswtg 60
gkgdyrbwnt msnwrmanrg artsstsgaa ttcccaagtt tgagagtaaa tattattagc 120
taaaaatggc agtcggaaag aataagagaa tgggcaaaaa gggagccaag aagaaggctg 180
tcgatccgtt cacacgcaaa gaatggtacg acatcaaagc gccggcgatg ttcacacatc 240
gaaatssts 249

<210> 65
<211> 362
<212> DNA
<213> Globodera rostochiensis

<400> 65
wcbcrbhdyb ytsgcrsnck tbdsbhcysy gcdwkmtnvk hscngdckty nyykkkvbmr 60
ntmsnwrman rgartsstsg tcaaccgtac tcagggaacg cgcatttcga gcgactttct 120
aaaaggccgc gtttacgaag tgtcactggg tgaccttaac agcactgacg ccgactttcg 180
aaagttccgc ctgatctgtg aagaggtaca gggcaagatt tgcctgacca actttcacgg 240
aatgtcgttc actcgggaca aactgtgctc tattgtcaag aagtggcaca cgctcattga 300
ggcgaatgtg gcagtgaaga ctaccgacgg tttcatgctc cgactctttt gtatcggtss 360
ts 362

<210> 66
<211> 128
<212> DNA
<213> Globodera rostochiensis

<400> 66
aatcaaatta agaagacgag ctatgcaaaa gcctctcagg tgcggatgat tcgtgccaaa 60
atggtggaga tcatgcagaa agaggtctct tccggcgatc ttgaangaaa gtagtcaaca 120
agcctgat 128

<210> 67
<211> 502
<212> DNA
<213> Globodera rostochiensis

<400> 67
gaattccatt aaaaaactaa acgaacaaat ctaaagatgg ccaccgaagt ggaggaaaat 60
gttcctacgg ttgacccatg ggtgctgtg gaggaagtgg gtggtgaaga gtcgatgcag 120
ttggtcagcc ttgacgttac cgaggtcaaa ctgttcggaa aatggtccct taacgatgtg 180
gaagtgtccg acatttcgct tgtggattat attgcggtga aggaaaaggc ggccaaatat 240
ctgccgcaca gcgccggccg ttaccaacag aagcgcttcc gcaaggccac ctgtccggtg 300
gtggaacggt tgtctttgtc aatgatgatg cacgggcgga acaacggaaa gaaactaatg 360
gcggtgcgca ttgtgaaaca ccccttcgag atcatcacct gctaccggag agaacccagt 420
ccaagtgttg gtcaatgctg tgataaacag tgggccccnc gaagattnca cacgtatcgg 480
acgtgcgggc actgttcgtc ga 502

<210> 68
<211> 519
<212> DNA
<213> Meloidogyne incognita

<400> 68
gcaaactttt atcaaataaa aaatttatat ttgccaaaca aatttatgaa taaaaattca 60
ttaatcatta aaactacatt taaaatatac tttttagaga atgtcgtcta aaatattctt 120
ttctcccctt tatgcatcta tctaaccaga cttggaagca atatggctaa tcaagtcaac 180
aatacggcag gaatacccaa actcgttatc ataccagcta accaatttaa caaaatgcgg 240
gttgagaacc ataagagcct cggcgtcgaa aatagacgaa tgagtgtcgc caagaaagtc 300
ggtagaaaca acctggtcct cagtatatcc aagaatccct ttaagctttc cttccgaagc 360
agtcttaatt gcattcttaa tagcctcctt cgttgctggc ttctccaaac gagcagtcaa 420
atcaacaacg aaaacgtttg ggcgtcggca cacgaaaagc catttccggt aagcttccca 480
tccaattcat ggattgacct ttccaacagc ctttgcagc 519

<210> 69
<211> 218
<212> DNA
<213> Meloidogyne incognita

<400> 69
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actgttttga agcgaaggaa agttagggct gctcagcgtg cttctctact caagaataaa 120
ttggagaata ttaagaaggc taaggttaaa acgcaagtta tctttaaacg tgctgagcaa 180
tacttgattg catatcgacg taagcaaaag caagagtt 218

<210> 70
<211> 293
<212> DNA
<213> Meloidogyne incognita

<400> 70
taagaaagca gggaattttt atgtcccaga tgaacctaaa cttgcttttg ttgtgcgtat 60
taagggaatc aacaaggtta atttaaattt gctataaagt ttaggatggg tttagacaat 120
tcttctcttt taatgctttc taactttttc aaaaaagtta tgattttatc acccattaat 180
ctacaaattc tttaatttat cagatccatc ctcgtcctcg aaaagttctt caacttttcc 240
gcttgcgtca aatcaacaat ggagttttca ttaaattgaa taaagctaca atc 293

<210> 71
<211> 422
<212> DNA
<213> Meloidogyne incognita

<400> 71
aatgcaatta agactgcttc ggaaggaaag cttaaaggga ttcttggata tactgaggac 60
caggttgttt ctaccgactt tcttggcgac actcattcgt ctattttcga cgccgaggcg 120
taagttttga ttttctaaga ttatatttaa ccttttttaat ttttcagtct tatgggtctc 180

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aacccgcatt ttgttaaatt ggttagctgg tatgataacg agtttgggta ttcctgccgt 240
attgttgact tgattagcca tattgcttcc aagtctggtt agatagatgc ataaagggga 300
gaaaagaata ttttagacga cattctctaa aaagtatatt ttaaatgtag ttttaatgat 360
taatgaattt ttattcataa atttgtttgg caaatataaa tttttatttt gataaaagtt 420
tg                                                                422


<210> 72
<211> 374
<212> DNA
<213> Meloidogyne incognita

<400> 72
atctgagcat aaggaaactt ggcctcaagc tatagagcag accgattatg tggcaccgac 60
tgagccagtt aaactggact tcaacgttcc gcttattagt gattgggctg ctgcttctga 120
gtggcctcaa gaagaggaag ctcaggttgc acctactgca ccaattggtc agccacagcc 180
tcaacagcag caaactcaac aaggaggtga ttggaactct ggtactagtg gatggtgaag 240
ggcaggaaaa ttgatagaaa gagaaattat tatggaataa atgtaatcaa tgttgttgtc 300
tgatttattt gttacatata caacaagttt tattttgttg tttatttaat aaaagttgtt 360
aattaaaaaa aaaa                                                   374


<210> 73
<211> 120
<212> DNA
<213> Meloidogyne incognita

<400> 73
tttttttttt tttttcttca tcaatatttt gaagtgaaga accagaagta gttgcattcg 60
agctttcaaa ttttgttttt tgattactct ttaaacaaga ttcaactgat ggatctactg 120


<210> 74
<211> 369
<212> DNA
<213> Meloidogyne incognita

<400> 74
gtctaaccaa tctagagcta ttcggttcgt ctgtctgttg attattagat gttgattgaa 60
cagcactagt ctctgatgta gttttcttca atctcatttt taagtgatgt agaggaagtt 120
tagaattctg attgctatcg tcttctttct cttcttttaa tggctttttc aatttatctt 180
cttccttttc ttgtccattc ttttcttcat tcttttcaaa aggctcagga aattttaatt 240
cagacccgct ccttttaact gctgtatcta aagaaaaccc tctaggcaac gtcccagttc 300
cactcaaatt caattttgtt aaatttttgc cagatctaag tccttcttcc ttttgaacga 360
attgaactg                                                         369


<210> 75
<211> 529
<212> DNA
<213> Meloidogyne incognita

<400> 75
ttttgttttt tttttttttt ttatcagaaa aaagtttaat cagaaaaaaa aattaaaaca 60
aatctaaata aggctctatt ctaagtttat atttttcttt tacataaacc gtcaaccctc 120
caagtttttc aatgcttgga ggttttaatg gatcctctgg taataatttg taggctagaa 180
aaaagtttgc agcaaaaagg aaaagcatca ttcttgctaa ggcttctcca gcacattgcc 240
ttttccccac accaaaagct attagctcgt cagctttttt taatttccct tcattgtcta 300
tataacgttc agggtcaaaa ttttggggat ttgggtatat ctttggatca aaaagaacat 360
ccgatacttg ggtatcata aatgtacctt taggcaacac aaactttcca acattcaaat 420
cttccaaggc taaatgcccc aaattgaaag ggactaaatt aacgagtctt aatgtttcat 480
taacaacagc atttgtataa attaatttag gtctgtgttc caaactaat             529


<210> 76
<211> 449
<212> DNA
<213> Meloidogyne incognita
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<400> 76
agtttttttt tttgaataaa agactttttt ttattaaaat ggcttcgcaa actgcaggaa 60
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gaaaggcaca acgacttaaa caagcaaaac aggaagcgca agctgaaatt gacaaatata 180
gagaggaacg tgaaaaacgt tttaaagagt ttgaacataa ttacctcggc gctagagatg 240
atattgctgc acaaataaag cgtgaaactg atgagacgct taatgaaatg actcgtagtg 300
ttgctgctaa taaacagcag gtaattgttc gtctacttca acttgtctgt gacattcgtc 360
cagaactgca tcacaattta caacttcaac ttaagcttaa tgaaaagcct gcctaatttg 420
tagttgattg attataaaaa tgaaattga 449

<210> 77
<211> 643
<212> DNA
<213> Meloidogyne incognita

<400> 77
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gaagcgtttg gccgctccaa agaattggat gttggacaaa ttgggtggag tttttgcccc 120
acgtcccatg tgcgggcctc acaagcttcg tgaatcgctt cctcttattt tgtttcttcg 180
taatcgtcta aaatatgcac aatcttataa tgaagctagg atgatttgca aacaacgtct 240
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ggtttccatt gagaaaactg gcgaagtctt tcgtcttctc tatgatgtca aaggacgttt 360
cattactcat cgcatacaaa aggaagaagg tcagcttaaa ttgtgcaagg tagtaaagca 420
agcgattggg ccaaaacaag ttccttatat tgttactcat gatgcccgta ctattcgcta 480
tccggatcca cacatcaagg ttgacgacac tgttgctgtt gatataaaca ctggaaaggt 540
tacagatcac attagatttg attctggtaa tgtttgtatg attactggtg gtcacaacat 600
gggacgtgtt ggtattggtt gacatcgtga acgccaccct ggt 643

<210> 78
<211> 584
<212> DNA
<213> Meloidogyne incognita

<400> 78
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aaataataaa ttggaatata ataaaaatga aattgagagg caaaaagagc aattaattcg 180
agatttgatt gcctccttaa cacgtgaaag gcaatattca cgagattggc aacaatcaca 240
acagcaacaa aatttcatta acagttttgg cccttcccca catttattcc cctcttcagg 300
cattgaatgg ccccaacaac aacaaaaaat atttttggaa gaaggggaag tagaagaacc 360
tttagaggaa aatgagaagg aaaaaagagc acaaactttt gttcgtttcg gaaagagagc 420
acaaacattt gttcggtttg gaaaaagggg acagactttt gttcgatttg ggagagattc 480
aaaacatcaa cataacttgt cagatcagaa gcagttaaaa actgacaaac aataaaaatg 540
atgaattatt taaaaatttt tttaatgatc ttttaattaa aatt 584

<210> 79
<211> 556
<212> DNA
<213> Meloidogyne incognita

<400> 79
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aggttgaggc ttctgatacc attgagaatg ttaaggcaaa aattcaagat aaagagggta 120
tcccgcctga tcaacagcgt ttgatctttg ctggtaagca acttgaagat ggacgaacct 180
tggctgatta taacatccaa aaggagtcta cacttcactt agttttacgt cttcgtggtg 240
gaaaggttca cggttcattg gctcgtgctg gaaagggtcg tgctcaaact cctaaggtcg 300
aaaagcagga acataagaaa aagaagcgcg gccgtgcttt ccgtcgcatt caatataacc 360
gtcgcttcac caatgttgct acttctgggg cgggacgccg tcgtggccct aactccaacg 420
ctgcataaga gaatggtcgt atcttgatga atgtatggtg atataatcaa tttaatacat 480
tcgactntat gaagttttct gttattcaag ataaatcttt ttgttgaaaa aaaaaccaag 540
tttgagatca gttact 556

<210> 80

<211> 424
<212> DNA
<213> Meloidogyne incognita

<400> 80
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cgatttggca attcgggatg gagttccata tccacctagg cctgcaatta ataatgttcc 180
tccatacctg aatatgttga ctcgaacgtt ttctgtacca aatgtaaatc agtacacggg 240
tgcaataggt ccttatcgac cagcaaatcc tgtttatact tattatagct ataaatgcta 300
ttttccgtat agaaattatc gaggctacac actgacggat gcttactggt acgaccgtta 360
ttattatttt tcgccaatat acaaacggtc aatgttccca attagattcc ggcattctga 420
ctac 424

<210> 81
<211> 89
<212> DNA
<213> Meloidogyne incognita

<400> 81
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caacanatta ccgcccattc tttgaccca 89

<210> 82
<211> 168
<212> DNA
<213> Meloidogyne incognita

<400> 82
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aaagacaaca taatttccaa ctttttcaat attatccttt ttaacggttt gattttgcaa 120
ctcgctccaa ttcgtccttc ttcttgatag catatgaatt gctcgaac 168

<210> 83
<211> 67
<212> DNA
<213> Meloidogyne incognita

<400> 83
aattcatcag ccagacattc agcaattgtt ttgatattac ggaaagaagc ttcacgagac 60
ccagtac 67

<210> 84
<211> 42
<212> DNA
<213> Meloidogyne incognita

<400> 84
taacacgacg aagaggcgaa acatcaacag cctgacgacg aa 42

<210> 85
<211> 429
<212> DNA
<213> Meloidogyne incognita

<400> 85
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tggattctct ccagtcaaaa tatgtataat ttcaaaagcg tgcttcacaa tccgaacagc 120
catcaacttt ttaccattgt tacgtccatg catcatcatc gaacaaacca aacgttcaac 180
aatcggacaa tgagcctttc gaaaacgttt gatttgatat cgaccagcac tgtgcggcaa 240
atatttggcc gatttgtctt taacagcaat ataatccact aaagaagcat cattaacttc 300
gatatcgctt aaagaccatt taccaaacaa tttaatttca ggaaaatcaa ttgtagtcat 360
ttgcatatcc ccttgtccac caggaacatc agttgcgccc caattatcat cagcgggtaa 420

accatctcc 429

<210> 86
<211> 435
<212> DNA
<213> Meloidogyne incognita

<400> 86
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aattttcttt tcatcatttt ttaatttaaa aaacatttta acaaattaca agaacaacaa 120
acataattgt ctccttttta ttataaaatt taaagtttaa taagttttaa aacattctcg 180
actggagtac gtgtacttag tgttttagaa aaggcaaaat tagtttgttg gtttgaagag 240
acaaattctt ttgcacaagt agcgagaaga tatcgagcag aatttggaat ggaacccccca 300
catatggatt tagttaaaaa attacatcaa cgttttctca atactggttc tgtttctaat 360
ggaaatactg aacattttga agttaatcca acaatggaaa catcgacatc ctcaacagag 420
ggtgtagcag atccg 435

<210> 87
<211> 501
<212> DNA
<213> Meloidogyne incognita

<400> 87
gttttttttt ttttttttta aacaaaatat cgagtcttta taagacaaaa ataaaagaca 60
aaagcaattt agttttatca ataaaattaa aaatagtcaa tgtctcgttt cactcattag 120
atttgtggcc ctaaagaggg ccgtttgggt ttggttgttg tacttcagct gccttccacc 180
aattgttcct tagccaccaa atccgtaaag agtacgtcct tggcgtttca acgcatagac 240
gacgtccatg gctgtgaccg tctttctctt ggcgtgtacg caataagtta ccgcgtcgcg 300
gatcacattt tcaaggaaga ctttcagaac acctcgagtc tcctcgtaaa tgagcccgga 360
aatacgtttc actccaccac gacgtgccaa tcgccggatt gccggtttgg tgataccttg 420
gatgatatca cgcaagactt ttcggtggcg cttagcgcct ccctttccaa gtccctttcc 480
gccttttact cgtccggaca t 501

<210> 88
<211> 270
<212> DNA
<213> Meloidogyne incognita

<400> 88
ggaagtgtgt ttaagataaa tggatgatta gaaataaaaa tgaattgatt aaaaattacg 60
ttagaataat aatggaatat ataaaaataa attggatgat ttaataaaaa aaaaaaagag 120
agaactagtc tcgagttttt tttttttttt tttttaanaa ttaacaattt atctcatttt 180
cctcttccat gaaaattaac aaaaagacga caacttaatc ccataattaa catcattttt 240
aagcttcagt cggcatgctt cgaataatgt 270

<210> 89
<211> 286
<212> DNA
<213> Meloidogyne incognita

<400> 89
caagcggttc ccaactcaat gttgttgcca tgatactcgt gaacaccagt tctcgccaac 60
atagaatagt actcaatctc actgcgtcta aggcttggag tattattcga aataataaca 120
agtttagcct ttccagaacg aagagtcttc aacgtctgct tgtagcccaa acaatacttg 180
cccgatttgg taaccatggc gagacgagca ttgatatttt ctgtggactt tttctgtttt 240
ccaacaacca ttgtaacgca aaattaaaat ctctttttta acaaat 286

<210> 90
<211> 391
<212> DNA
<213> Meloidogyne incognita

<400> 90

AKK110P1

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tcctaggcct gcaattaaca atgttcctcc atacctgaat atgttgactc gaacattttc 180
tgtaccaaat gtaaatcagt acacgggtgc aataggtcct tatcgaccag taaatcctgt 240
ctatacttat tatagctata aatgctattt tccgtataga aactatcgag gctacacatt 300
gacggatgct tattggtacg accgttatta ttatttttcg cctatataca aacggtcaat 360
gtttccaatt agattccggc actctgacta c                                391
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<210> 91
<211> 131
<212> DNA
<213> Meloidogyne incognita

<400> 91
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caacaaatta ccgcccgttc ttcgacccac gcatcagcgc atcattttca agaccttatg 120
attacacatc a                                                      131
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<210> 92
<211> 571
<212> DNA
<213> Meloidogyne incognita

<400> 92
ttggtgcgac aacaaaaaaa ttttatttat tttttaacaa cagaaaaata tactttttaa 60
tttttaatat ttttccatga ttcaacagcc atactttcct cattttaata cttcttaaac 120
cctcaaaaaa ttcatttatt gacgaccagc agcaggttgt tgctgctgtt gttgaccacc 180
accccccttgc gcttgacctt gctgttgctg tcccttcacg tcaacaggca aattgagttg 240
caaataatca accatctcct tagtctcttg atcaacacta atagttggat gttgagaagc 300
atcaagatag gaaacttctg gaacccaatt atcacgacgc tcacgctctt cttcttgcaa 360
tttaatagaa attccacgaa caggtccttt ttcgatacgc ttcatcaaat gggtaataaa 420
accagcaatt tgattacgca tccgtttgct aggaataaca gcaatttcct cacaaattcg 480
tttgttcaca tgaaaatcat aagtcaagcg tgtataatat ttgtcaataa taacacgaga 540
tgctttcttg acagttttga gagaaccgat t                                571
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<210> 93
<211> 671
<212> DNA
<213> Meloidogyne incognita

<400> 93
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accaatacgc gtgctggtta ctggagcagc tggtcagatt ggttattctt tggttattca 120
aattgcaaag ggtgatgttt ttggaaagga aacgcccatt gttctggtaa tgttggatat 180
tcctccaatg gccgaagtgc ttaaaggagt ggaacttgaa ctttacgatt gtgccttggc 240
gaatcttata gctgtcgagc cagtcacgac tgaagaggca gcgttcaaag acattgatta 300
tgcttttctt gttggtgcaa tgcctcgaaa ggaaggaatg gaacgaaagg atttacttgc 360
tgctaatgtg aaaatattta aatcgcaagg attggctcta gcaaaatatt caaagccaac 420
tgttaaggtt ctggttgttg gaaatccagc aaatacaaat gcttttattt gtgcaaaata 480
cgcagcagat aaaattccag caaagaatgt cagcgctatg actcgtcttg accataaccg 540
tgcaattgcc caaatagctg ctcgttgtgg ggttgactgt ggatctgtga agaaagttat 600
aatttgggga aatcattcaa gtacccaatt tcctgatgtt aaacatgcta aagtaattaa 660
aggtggcacg g                                                      671
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<210> 94
<211> 289
<212> DNA
<213> Meloidogyne incognita

<400> 94
ggctgtaaat gatgtgccgt ggatacagaa tgaatttatt tcgaccgtcc aaaagcgcgg 60
agctgttatt atcgaaaaac gcaaactgtc cagcgcaatg tcggcagcaa aggcggcatg 120
tgatcacatt catgattggc actttggaac aaaagatggc gattgggttt ctatggccgt 180
tccttccgat ggttcttatg gaattccgga aggtttgatc ttctcatttc caattacaat 240
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tgatgcanaa acgcgtgact ggaaaattgt acaaagatta gaactcgat 289

<210> 95
<211> 262
<212> DNA
<213> Meloidogyne incognita

<400> 95
aatttaactt ttctaaccaa aacttttatt tttgtctttg atgtctactc aagtaccgat 60
acgcgtgctg gttactggag cagctggtca gattggttat tctttggtta ttcaaattgc 120
aaagggagat gttttcggga aagaaacgcc catcgttctg gtaatgttgg atattcctcc 180
aatggccgaa gtgcttaaag gagtggaact tgaactttac gattgtgcct tggcaaatct 240
tatagctgtc gagccagtca cg 262

<210> 96
<211> 323
<212> DNA
<213> Meloidogyne incognita

<400> 96
aagacattga ctatgctttt cttgttggtg caatgcctcg aaaagaagga atggaacgaa 60
aggatttact tgctgctaat gtaaaaatat ttaaatcgca aggactggct ctagcgaaat 120
attcaaagcc aactgttaag gttctggttg ttggaaatcc agcagataca aatgctttta 180
tttgtgcaaa atatgcagca gaaaaaattc cgacaaagaa tttcagcgct atgactcgtc 240
ttgaccataa ccgtgcaatt gcccaaatag ctgctcgttg tgtggttgac tgtgggtctg 300
tcaagatagt tataatgtgg gga 323

<210> 97
<211> 717
<212> DNA
<213> Meloidogyne incognita

<400> 97
aatattttta acaaacgatg taacagaaaa acaaagtttt tttaacaaat tttcttgaac 60
cttatttttt ttcaaaacat ttttttattt aaatttaaac ctctcttcat ttctcttaaa 120
cactttcctg aactggaggt tcataagcat ctggacgact ttcaataact tctccacttg 180
ctgtagttat agcaacttgt ccaccaccac ttccagcacc ctctccatgc atatccaaaa 240
gttttccaag ttcaaatttt ggttttttca aaatttttac ttttcgaata taaacgtctt 300
gaagtggata gaaataagaa caagactttt caatgtcttt tccaatagaa tcaggaatta 360
atttgctgac aacttcttta agatcgcatg aagaaacctc gcgatgaata atctcaacca 420
tcctagcacg aatttgacgc acttgagacg attttgcata actagtcttt ttcacttggt 480
ttggagcttt ctttgtgaag ccaatacaga acaatcgaag caaataacca tcagttgttt 540
tgacagcaac atttgcttca attaaagtat gccacttttt gacaatagaa caaagcttgt 600
ctcgagtaaa agtcattcca tggaaattgg tcaaacaaac tttgccttga acctcttcac 660
aaataagtcg aaatttgcga aagtcagctt cggtgttgtt cagatcacca agagaaa 717

<210> 98
<211> 758
<212> DNA
<213> Meloidogyne incognita

<400> 98
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taacaacaaa caaaaatggg cgagcaagac aaaaagaaag ctggcggcgg cgatggtggc 120
aaaaagaagg atggcttcga tgccaaaaag tttgcgattg atttggcttc tggaggaact 180
gccgctgcgg tttctaagac ggctgtggcg cctattgaac gtgtcaagtt gttgctacag 240
gttcaagacg cttctcagca catcgctgcc gataaacgct ataaaggaat aattgatgtg 300
cttgttcgtg tgcccaaaga acagggagtc cttgcttttt ggcgtggtaa tttggctaac 360
gtgatccgtt actttccaac gcaagctctc aactttgcgt tcaaggacac ttacaagagg 420
atcttcatgg aaggtgttga caagaacaaa cagtttggca aattcttttt gatgatgctg 480
tttatgagca aaaatttcct tggttggaat agacctaaca gttgaagagt atcttgccct 540
ctgtgatacg tatacaacac tctcttcaat tggagattca atgttgagtg gagatgctga 600
tagtaatcct ctgttacaat cacttaacaa ctcaatcaat tccaatgcca ctgctcagaa 660
ttataactcc tcaacaattg gccgaagcta aaaactacgt ttcaacatgc tacagctact 720

tcaaaatcga aacagattgt tttaaacgtt tgaaattt 758

<210> 99
<211> 154
<212> DNA
<213> Meloidogyne incognita

<400> 99
ttgagttcgt tggcacattt gttgtgttac aaaacgaaaa ttattgggaa cgggtttcag 60
tgcctattct cgcaggttat tggcacttca cacatttgta ccaataacaa cgttaccgtt 120
tataatcaaa ctgttcctca aagttatgcc catt 154

<210> 100
<211> 125
<212> DNA
<213> Meloidogyne incognita

<400> 100
ttcagaatac tcaaggtctt atattcgttg ttgatagtaa cgacaaagag cgtattgttg 60
aagctcgtga ggaattgatg cgtatgttgt ctgaagacga acttcgcgat tctgtactcc 120
tcgta 125

<210> 101
<211> 219
<212> DNA
<213> Meloidogyne incognita

<400> 101
cttgccgaat gctatgaacg ctgctgaact tacagacaaa cttggacttc acacgctgag 60
aaatcgtaac tggtatatcc aggctacttg tgccacttca ggagatggtt tgtatgaagg 120
tttggactgg ttgagtaacc aattgaagaa tcaaggttaa atgagtctaa ataaaaatgg 180
agaggggaaa gaggagaggt taattttta aggaaaaaa 219

<210> 102
<211> 473
<212> DNA
<213> Meloidogyne incognita

<400> 102
gtttttttt tttttttta aattccaagt tttcttccaa atgagagaat agggagaatg 60
atgggggaaa aaataggagc aagccaaaaa gccaaaaaaa aattttttt ttaaatgatt 120
tttgtaaatg tgtgaaaagg tgtgtgtcaa ttgtagagtc aaatgtcgtt gccttccttc 180
cactaaaatt tctctttcct ttcttttctc ttctaaaatt ccttcaaagt cgatccaacg 240
aaatttcagc ctcctctgga tattccaact cccaaatacg cttcaaatgt ttgcctttaa 300
cgtcacgagg agtaccaaat ccagtcatca acttttgaga gtctccctta ttccaaccgg 360
cctgggatgg aattatcgtt tctgacttct tcatatcttc atatggaagt tcgccagact 420
ccgcctcgta tgttgtgttc cttggcgttc caaaacctgt catgcccgct tgc 473

<210> 103
<211> 114
<212> DNA
<213> Meloidogyne incognita

<400> 103
ttggaccgtt aggattgtcg ccaaagaaaa ttggagaaga cattgcaaag gcaacacaag 60
actggaaagg cttaaaggtt acttgcaaat tgactatcca aaaccgaatt gcca 114

<210> 104
<211> 255
<212> DNA
<213> Meloidogyne incognita

<400> 104
ccgcttctcg aattgtgaag gaattgaagg aacctcaccg agaccgcaaa aaagtcaaac 60
acgtaaaaca cagtggaaat ttgacgatcg agcaaattat caacattgca cggcaaatgc 120
gacctcgttc aatggcgaaa aaaattggaa gggactgtta aggaaattct tggcactgca 180
caatctgttg ggtgtactgt tgatggacaa catccacatg atattgttga tgcaatccga 240
agtgggaaaa ttgaa 255

<210> 105
<211> 571
<212> DNA
<213> Meloidogyne incognita

<400> 105
tttttttttt tttttttttt tgtcaacaat aaatttactc agaaaaatca tttaacaatt 60
taacacacat ttttaattcc ttaatactcc aaaaaacttc tcttctttat tccctcttat 120
tctcccaatt catttaaagt ttcagttttg tgcggcgcca atgacgacgt tttgcattat 180
agcgtatacg actgccagtt ttcattcgaa cccattgcgg cagcggtcga ttttgtttag 240
cagccttagc cagcttgcgc ttgataataa acgttttgtg tgcagccatt aaattgttga 300
ctttatccaa aattgttttt ttgaaggcaa taaacaaatt taatttttct gctcaacaag 360
tccatagcag ctcatctggt caacaatctc cctcatgcgc ctcagtctcc agcgcttcct 420
cttatgaatg tcaaaaacag cagcaacaac ccccagcaga accttgtgga ccttctttgg 480
aagttcatca atctggtcat tcaacaacaa cccttccatc tccatgtnct ttattacccc 540
ctccctcttc tttacatcct ataaatcatc g 571

<210> 106
<211> 235
<212> DNA
<213> Meloidogyne incognita

<400> 106
tgctttattt tcaattcttc aaccaaaaat taaatcttcc cttattttaa ttacaattcc 60
aattttagca gcattagccc caactacttt agctgctaat aaaattgttt atgaggatgg 120
agatagtgat ggacttgata tggctaaaag tattttaaat tgaataaagg aaaaagaagc 180
attttaaaga aaattagatg gaaatgctga agaaagaaaa aaattattta ttttt 235

<210> 107
<211> 702
<212> DNA
<213> Meloidogyne incognita

<400> 107
ttttttcaaa aaataattcg aattttgttc ttttttattt tgctacaaat aaaatttaaa 60
tttgaaaaaa aaaaaaaaaa aaaaaaaaac tcgagaagaa atccttgccg aaattgacgg 120
ctctcaaatt gaggagtatc aacgtttctt cgatatgttt gaccgtggaa agaatggcta 180
tattatggct actcaaattg gggtaattat gaatgctatg gaacaagatt ttgatgaaaa 240
aactcttcgg aaattaatcc gaaaattcga cgcagacggc agcggcaaaa tcgaattcga 300
cgaattctgc gctttggtat acactgtggc gaatactgta gacaaggaca ctttgcggaa 360
agaattgaga gaagcttttc gtctctttga caaagagggc aatggttaca tctctcgtcc 420
aacactcaaa ggattacttc acgaaatcgc cccagacctc agcgataaag acttggatgc 480
cgcagtagac gagatcgacg aagacggaag cggaaaaatt gaatttgaag aattttggga 540
gttaatggct ggagagactg attgaaattt taattagaat gactagaaaa ttgactaaaa 600
tattttgcca ttaaattttg gaaagtgcca aaaattgcct ttctgagaat ttttattttt 660
aacgtctaaa taatgaataa aatggatata aaaaaaaaaa aa 702

<210> 108
<211> 423
<212> DNA
<213> Meloidogyne incognita

<400> 108
aaaattaaaa taaaagacaa acaaataaat ataaattaaa taaataatat ttaaataaac 60
acacaaataa actctccaaa cataattttt ttaaatttta ataacatttt gtcccatttg 120
agaaagaaaa tgccaaagga gatgaagaac ttgttgaaga aaaaagttca aaaatatcaa 180
ctcctccatt tgtcgtcaca ttttctttca ttattccatt tgttgtaagc tcagtaactg 240

ccccaattgt tgttgtagtc catggagaga aagcactttc cccattcgaa aatgttgaac 300
caaattggtc aaattgttgc tgttgttgac ctcgaagttc gttagaaaca gaacgaaata 360
aattatgagg ttgttgttgt tcctgacgtt tttgattgtc tggagctggg tgaggatcac 420
caa 423

<210> 109
<211> 994
<212> DNA
<213> Meloidogyne incognita

<400> 109
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tagaacaggt gctggcgatc ttgtcacaac ccctggacct cttcataaac aaattgaaag 120
gtcaaaacta gccaagccga aattcaagcc tttaaaacgt tcaagagaag agcaaaaaga 180
tgaaattgaa cttgtcgatc catcgttaaa gggcaaaatt attattaaag caaacaaaaa 240
attggaaaaa gatgttgtgt tcaatgagga tggagaatct gataattctg aagaaattga 300
agaagaagaa gaagacggca atgaaaagtt ggatgttgat caattagtat caaaacattt 360
ggaagattta gatgaactaa aattggatga tggcgttgaa aatgtgcgaa agataataac 420
gaaattcaga taaaaataac aaagaaagtg ttataaataa agctgagttt gccgatatcg 480
acccaaaaat tgttgatctt tttacagaaa ttggtcaagt tttaaagaaa tatagaagtg 540
gacgtattcc caaagctttt aaagttattc caactttggt tgattgggag aaaattatcg 600
aattaactcg cccagatgat tggtcggcag ctgcaatgtt acatgctacc aaaatatttg 660
cttcaactgc taccccctact caatgccaaa ggttttataa tttgattttg ttgccacgta 720
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caatttttct cttcgagaag ctgttgttct tgcttctatg cttcgtaaag cctccatccc 900
tcaattacac gcggccgcag cattgttgag tatttcttgt ttagaatata cttcttcaag 960
ggcttatatc cttcaagcat tgatagaaaa gaat 994

<210> 110
<211> 476
<212> DNA
<213> Meloidogyne incognita

<400> 110
tttaaacact taaaaatacc ttcaaattta ttttagaacc tttttgccat taaaaaaaat 60
tttatttcga aaaaatggct gagaatatag aagaaatcct tgccgaaatt gacggctctc 120
aaattgagga gtatcaacgt ttcttcgata tgtttgaccg tggaaagaat ggctatatta 180
tggccactca aattggggta attatgaatg ctatggaaca agattttgat gaaaaaactc 240
ttcgaaaatt aatccgaaaa ttcgacgcag acggcagcgg caaaatcgaa ttcgacgaat 300
tctgcgcctt ggtatacact gtggcgaata ctgtagataa ggacactttg cggaaagaat 360
tgagagaagc ttttcgtctc ttcgacaagg agggtaatgg ttacatctct cgtccaacac 420
tcaaaggatt actccacgaa atcgccccag acctcagcga taaagacttg gatgcc 476

<210> 111
<211> 189
<212> DNA
<213> Meloidogyne incognita

<400> 111
cgaagacgga agcggaaaaa ttgaatttga agaattttgg gaattaatgg ctggagagac 60
tgattgaaat tttaattaga gatgaataaa aaattaacta aaatattttg ccataaaatt 120
ttggaaagtg ccaaaaattg cctttttgag aatttttatt tttaacgtct aaataatgaa 180
taaatggat 189

<210> 112
<211> 164
<212> DNA
<213> Meloidogyne incognita

<400> 112
ttgaggaaat ttaatttttt aaacaaatat aataattacc aaacaacaaa aaagaatccc 60
aaaaacaaca tttttaaatc aaatgacaga catatatttg caataacgat gtgtggattt 120
tctttttttt taaataatta acatcttaag cctgctattt cttc 164

<210> 113
<211> 539
<212> DNA
<213> Meloidogyne incognita

<400> 113
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taacaccaac agccacagtt tgacgcatgt cacgaacggc gaagcgtcca agaggagcgt 120
agtcagtaaa agcctcaaca cacattggct tggttggaat taagtcgaca ataccagcat 180
ctccagtctt caaagccttt ggattgtctt caaccttctt tccagttcga cggtcgacct 240
tctctttaag ctcagcgaac ttgcaagcaa tgtgagcagt gtgacagtca agaacaggcg 300
tgtagccagc agcaatctgc ccaggatggt tcatgatgat aacctgagca gtgaattgct 360
tggtctcctt tgctgggtca ttcatagagt cagaagtgac tgaaccacgt cggatgtcct 420
tgacagagat gttcttaacg ttaaatccaa cattgtcttc aggaacagct tcagggagag 480
actcgtggtg catctcaaca gatttaactt cagtagaaat tccttcagga gcaaaggta 539

<210> 114
<211> 314
<212> DNA
<213> Meloidogyne incognita

<400> 114
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tccgtcgatt tactgagatt ggttcttcta aatttgccca tcccgctttt gttccaagcc 120
cggagaatct tgaaagagta aggaaatgtc cagttttggt tgttggtgct ggtgggcttg 180
gatgtgaaat tttgaaaaat ttggccttat caggatttca aaatattgaa gttattgata 240
tggacacaat tgacctttca aatctcaaca gacagttttt gtttcgtgaa cacgatgttg 300
gcttatacaa agca 314

<210> 115
<211> 200
<212> DNA
<213> Meloidogyne incognita

<400> 115
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gacttgactt ttatgggcaa ttttcaatta taatttgtgg actagattct attgatgctc 120
gaagatggtt aaacgccaca gtgtgttctt tggtcgaatt tgacgaagaa aacaagccac 180
ggccaggcac aattattcca 200

<210> 116
<211> 471
<212> DNA
<213> Meloidogyne incognita

<400> 116
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gagccattgt tgattgtagg aaaggacaaa tttgctggaa tggatattcg catccgtgtc 180
aaaggtggtg gtcatgttgc acaaatttat gcaattcgac agtcaattgc taaagttttg 240
gtggcctatt accagaaaaa cgtggatgag caaagcaaga aagaattgaa ggatcaactt 300
gttgcttatg atcgtaattt gcttgttgcc gatccgagac gtcacgagcc aaagaagttt 360
ggaggacctg gtgctcgtgc tcgttatcag aaatcttatc gttaagaagt atgaaattat 420
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<210> 117
<211> 593
<212> DNA
<213> Meloidogyne incognita

<400> 117
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AKK110P1

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ctccatctgc tgatgctcca atgtttgtgg ttggtgttaa tgaggacaaa tatgatcctt 480
ccaagcatca tatcattagt aatgcttcct gcactactaa ttgtcttgct cctcttgcga 540
aggttataaa tgacgagttt ggcataattg aaagttgaat gactactgga cac 593

<210> 118
<211> 576
<212> DNA
<213> Meloidogyne incognita

<400> 118
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tgagggggaa gtaaaatgaa agaagggaga gagatatgaa ttggaggttt ttttgttaaa 180
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atctccatta tcgctgattg caagggcatg ggcgttttcg agaccctttg caaagctatt 360
agcccttcct gtgttcatat ccattacgaa aacttgggat tctaattgac tgccttgatc 420
ttgattggtg acgccgacga ggaagtgttc tttctctcgg atagcaaaga ctcgcccaat 480
attttcagcc tttgtgaaga aagtgcctgt ggggacgtaa gcacgtctat gttggtgttg 540
agcgccttct aatccagcag aaaagcattg aatacg 576

<210> 119
<211> 559
<212> DNA
<213> Meloidogyne incognita

<400> 119
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tcaaacttca ttattcggct attacagtaa ccatctgcca cgaaaaactc tcctgtactg 180
gcaatagcaa cgtctgtagg tttgcaaaaa tgtttgtcat ctgtccctgg aacaagcttt 240
tcgcccaaac tcataattaa tttaaaatcc ttgtcaagtt tgtggacttg atgacttcca 300
acgtcagtaa cccaactatt gccgtgggca tcgattgtta gtccatgagg catgtaaaac 360
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gtgtttgaaa tgatgcccag ggatctgttt aggtggttgt tctcatcaaa cgaaaattca 480
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cccggagctt gcccaatat 559

<210> 120
<211> 366
<212> DNA
<213> Meloidogyne incognita

<400> 120
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agttgaagga gaactccttc ttaattggat taagaaagtg acaggcgaaa atattgctat 180
aaacggaact agggaaaatt ttgtgaaaca attgaaagat ggaactctgc tctgcaaatt 240
tgctaacaaa attgtgccaa attcaatcac aaaggcacag gcaaaaccga acagcacatt 300
ccaatatatg agcaatttgg agctgttctt aacatttatt tcaagccaag gagtccctag 360
ggagga 366

<210> 121
<211> 661
<212> DNA
<213> Meloidogyne incognita

<400> 121

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ggtactggag accaagtgcg ccttcgtgtt taaagatggg aaattgaaag aattttggtt 180
aaacataata aaaagacatt ttatggcaat aaaaaaatgt caaaaaagct tgtcttttaa 240
atattttggc aaaacatttt actttcacaa aattttaaaa taaatttatg aagattgttc 300
cgtcactttc atcatttccg atcgaccttt gttgttttct aagttcgttg gccaaagaaa 360
ggatatgtaa aattgaatta tgaataaaaa taaatcactc aatcagaggc attgttagtc 420
tctcacttcc tcctctttac ccattggcta accagcttta aggatttttt ccataagttc 480
aaggtgtacg taaatcgaat accgactgtg gtatcttaat ttttccatga aattctccaa 540
taaaaaaaaa ttttttttat tttttttcca taatgctatc tatatttttt gcttttaatc 600
tttttggct atcaggcttt aaaatagtaa atatacttat attaatattt tatttccttt 660
a                                                                 661
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<210> 122
<211> 173
<212> DNA
<213> Meloidogyne incognita

<400> 122

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gggaaattgc tcagtcaatt tgggaagcct gactccagtg aaacacccaa aaatggagaa 120
ttccttgtac cacactctct aaccctcatt gaagatctca acttactttg tgt        173
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<210> 123
<211> 584
<212> DNA
<213> Meloidogyne incognita

<400> 123

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gtccccacag gcactttctt cacaaaggct gaaaatattg ggcgagtctt tgctatccga 120
gagaaagaac acttcctcgt cggcgtcacc aatcaagatc agggcagtca attagaatcc 180
caagttttcg taatggatat gaacacagga agggctaata gctttgctaa gggtctagaa 240
aacgcccatg cccttgcaat cagcgataat ggagatattt atgtttcaca aatagaaccc 300
aaccaaattg taaaatttag tatttcgaca aacgaaaatt gagaaaaaaa aaaaaaaagc 360
tcagaaacgg gaagaatttt caagaaaaaa tttttttacc aaacaaaaaa cctccaattc 420
atatctctcc cttctttcat ttttccttcc ccttctcccc aaaaattaca aaaaatttta 480
ttgtgcacaa aaaaatgggc gggcgggcga atggctgggc aaaggatggc gataaatctt 540
ttaatttttg aaaaaaaaaa aaagaattcg aattatatgg ccta                  584
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<210> 124
<211> 650
<212> DNA
<213> Meloidogyne incognita

<400> 124

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ctcttctcaa gaagtacttc acgaaggaag ttatggacca gtgtaaaggg ctcaaaacta 180
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agaattatac ggaaatgcat gacaaagtta aaggggtttt tgagcagctt aagtctgatg 540
ctgagcttgg tggcacctat tatcctttgg agggaatgac caaagaggtt caaactcaat 600
tgatcaagga tcacttcctc ttcaaagaag gagaccgctt tttgcaagct            650
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<210> 125
<211> 1013
<212> DNA
<213> Meloidogyne incognita

<400> 125

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gttttgaatc aaataattaa attttaaatt atttaaacag ctacacgagg cctcagcctc 180
ccccgttgca ttcaaattgg tcggcacggt tggcgatgat aattttattt tttaggtaat 240
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accaaacatc cggaatacaa ccacgaagtt aacattgacc aaagcgaaat tcctttgcaa 960
tctggtacaa acaaattcgc atcccaaaag ggaatgacca gcttcggtac aaa 1013

<210> 126
<211> 80
<212> DNA
<213> Meloidogyne incognita

<400> 126
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cttaccaaat gggatcaaat 80

<210> 127
<211> 585
<212> DNA
<213> Meloidogyne incognita

<400> 127
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agaaccgtaa atcacaagga atggtccgtc tccaatccgg aacaaaccgg gtcgcctcgc 120
aagcgggcat gacaggtttt ggaactccaa ggaacacaac atacgaggcg gagtctggcg 180
aacttccata cgaagatatg aagaagtcag aaacgataat tccatcccag gccggttgga 240
ataagggaga ctctcaaaag ttgatgactg gatttggtac tcctcgtgac gttaaaggca 300
aacatttgaa gcgtatttgg gagttggaat acccagagga ggctgaaatt tcgttggatc 360
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cgacatttga ctctacaatt gacacacacc ttttcacaca tttacaaaat acattaaaaa 480
aaaatttttt ttggcttttt ggcttgctcc tattttttcc ccccatcatt ctccctattc 540
tctcatttgg atgcaaactg gaattttaaa aaaaaaaaaa aaaaa 585

<210> 128
<211> 287
<212> DNA
<213> Meloidogyne incognita

<400> 128
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agccaaatcc aaaactttac aaaatgcaaa tttttgccag taatcatgtt gttgctaaat 120
cgcgtttctg gtactttact agtatgttgc gtcgtgttaa gaagactaac ggagagattg 180
tttcgtgtca ggaggttttt gaaaagaaga taggctctgt aaagaattat ggaatttggc 240
ttcgttatga ctctcgaacc ggtcatcaca acatgtaccg tgaatac 287

<210> 129
<211> 175
<212> DNA
<213> Meloidogyne incognita

<400> 129
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caaataatca aggttcaacc gatcaaggct gccgattgca aacgtactgg agttaaacag 120

ttccacaact cttcaatcaa gtttcctttg ccgcatcgtg tgaatgacaa acgtc 175

<210> 130
<211> 599
<212> DNA
<213> Meloidogyne incognita

<400> 130
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aaaggttcac cttctaagta aggattgtag cggctgtatg attgatgttg cttttgttgg 120
ggagcaatag aacgcttgcg tcgccgaggc tcctcagccc tagtaacgtg aaatttcttt 180
gcaatcatcg atttgtgtag tccatttttg gctaagacct gttctaagtc ttgttcatat 240
tgttcagaat tgctttttga ttgacagtta aacatgtgtt cttggtcaca aaggcattgc 300
tgattggcct ggtagctacg cgagaaatcg gcggtgttat caaactcctc caaacatcca 360
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ttgtcactct taatctcttg gcggtgtgaa aattcagatt ctggatggag ttgttggtct 480
ccttcaccgg cacctcctgt cataaattta tgtccaaacg caatgggccc ggaagcactt 540
tcaatgtcac gagaaatcaa gtcgattaat tgtgaatgcg gaaatatagg ctccccaga 599

<210> 131
<211> 466
<212> DNA
<213> Meloidogyne incognita

<400> 131
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tcctgtaggt ggtaggagca ggctgctgca gccatatatc attgggatca gcattgtcag 420
gataggtgat gcccagatag ttcaagatct tctgataacg ctgggg 466

<210> 132
<211> 266
<212> DNA
<213> Meloidogyne incognita

<400> 132
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tggaattgga agcgccgttg aagatttgtg gcgatattca cggtcaatac aacgaccttt 120
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atgtggatag aggaaagcag agcttggaga cgatttgttt gctgttggcc tacaagatca 240
aatcccccga aaattctttt tgctga 266

<210> 133
<211> 308
<212> DNA
<213> Meloidogyne incognita

<400> 133
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atattttgtt gccatggagg tttgtcacca gatttgcaga atatggagca aattcgaaga 180
attatgcgac cgacggatgt gccagataca ggtcttctct gcgaccttct atggtctgat 240
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ccagatgt 308

<210> 134
<211> 335
<212> DNA
<213> Meloidogyne incognita

<400> 134
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tggagaatgc aatagctaaa cttcgaaaaa atcctgatct tccattaaag tgggatactt 240
ttataagtgt ttcgcctcaa caacagcaac aacaacagac gagaatgaat actggagaaa 300
atgcagtttc ttataaacaa agcactccta tcgaa 335

<210> 135
<211> 506
<212> DNA
<213> Meloidogyne incognita

<400> 135
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tcggctnttt gccaatccct tgcgtc 506

<210> 136
<211> 230
<212> DNA
<213> Meloidogyne incognita

<400> 136
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ttttgttggt ttttaattgt ttatttttgc tactaatttt ccttctaatt gccgcctacc 180
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<210> 137
<211> 216
<212> DNA
<213> Meloidogyne incognita

<400> 137
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tcactcgctg tttgtaccaa ctctactagc tgtaattcgt ccttagctgt gccgttaatt 120
tctagtgaat cggaagaaag tgatgaacaa caaaagacgg gggaatggac aaatctaaca 180
ttattaatta tttattctca tgattgtaaa ttgcat 216

<210> 138
<211> 395
<212> DNA
<213> Meloidogyne incognita

<400> 138
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ctctgttctc gttatgtgtc acactcgcga acttgctttt caaatttcaa aggaatatga 180
aagatttagc aaatatatgc ccggaactaa ggtttcggtt ttctttggtg gtatgccgat 240
caagaaggac gaggagactt tggctaagaa cactccgcac attgttgttg gcactccagg 300
gcgtctgctg gcgttgggac gtacaggaca attgaagctg aaaaacatca aattcttcgt 360
tttagacgaa tgtgacaaaa tgattgggga cgctg 395

<210> 139
<211> 591

<212> DNA
<213> Meloidogyne incognita

<400> 139

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gactggcagt cacttactga agtcaaggaa atgggtctta tgctgttgaa ctgctcctgt 180
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gaatttgttc gaattgcggt aatggattat attcctgcaa caatttacat gccgaatggt 480
aacaacatat attggccatc gatcgatgac gcgataagaa cggcagctta tcggggtgtg 540
aaagttgacc tttggtgagt ctgtggcccc atttgaatga acgagcgatt t          591
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